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DETECTION OF CHANGES IN THE CONCENTRATION OF UNBOUND NORADRENALINE IN ADRENERGIC NERVES

BY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Detection of Changes in the Concentration of Unbound Noradrenaline in Adrenergic Nerves, submitted by Margaret M. Chambers in partial fulfilment of the requirements of the degree of Master of Science.

ABSTRACT

An in vivo enzyme assay method for monoamine oxidase (MAO) was studied with a view to the possibility that it may be used to obtain an indication of changes in the concentration of free noradrenaline (NA) in sympathetic nerve endings. A synthetic substrate for MAO, kynuramine, is converted by deamination to a fluorescent product, 4-hydroxyquinoline (4-OHQ). In vitro tests using isolated guinea-pig liver mitochondria as a source of MAO, demonstrated that NA can compete with kynuramine for deamination by MAO and reduce the output of 4-OHQ in proportion to the concentration of NA present. In vivo tests, using isolated perfused guinea-pig hearts as a source of in situ MAO, demonstrated that a steady state of 4-OHQ output can be obtained when kynuramine is perfused through a tissue containing adrenergic nerves. In addition these tests also showed that simultaneous infusion of NA will cause a reduction in the output of 4-OHQ, presumably due to uptake of NA into the nerve ending to compete with kynuramine for deamination by MAO in the mitochondria of the nerve ending.

Desmethylimipramine (DMI) which blocks uptake of NA by adrenergic nerve endings, reduced and nearly prevented the inhibition of 4-OHQ output by NA, thus supporting the hypothesis that the deamination occurred in the nerve endings. Reserpine, which causes an intracellular release of NA, also caused a reduction in the output of 4-OHQ which was not prevented by DMI. Further evidence for the validity of the method was obtained by showing that the observed fall in 4-OHQ output is not due to vasomotor activity, as dopamine, tyramine and phenethylamine, which have low vasomotor activity, caused inhibition in the output

of 4-OHQ in the order of their reported uptake into nerve endings.

Electrical stimulation causes a reduction in 4-OHQ output, suggesting an increase in the intraneuronal pool of free NA.

6-Hydroxydopamine (6-OHDA) treatment causes a reduction in both the NA content and the MAO activity of heart and vas deferens tissue, indicating that the MAO involved in the deamination of NA is associated with sympathetic nerve endings.

The conclusion drawn is that the experimental procedure tested in this thesis can be used to detect changes in the intraneuronal pool of free NA.

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To My Parents

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INTRODUCTION

This investigation was carried out to observe changes in the size of the pool of unbound noradrenaline in sympathetic nerve endings as a result of various treatments. The observations were made in an attempt to provide evidence for or against the intracellular theory of release of noradrenaline from sympathetic nerve endings.

Studies on the storage of noradrenaline have shown that nearly all the catecholamines in adrenal medullary homogenates can be sedimented in a layer corresponding to that of the dense granules which Blaschko and Welch (7) and Hillarp, et al. (55) showed to be the storage compartments for noradrenaline in this tissue. However, differential centrifugation studies on peripheral tissues have shown a considerable proportion of the total noradrenaline content of the tissue to be in the soluble supernatant fraction, rather than in the granular fractions. It is difficult to interpret whether this observation is an artifact caused by the breaking of the storage particles during homogenization and centrifugation, or whether the observed soluble noradrenaline represents a physiological pool of noradrenaline, normally present in an unbound form, in the cytoplasm of the nerve cell. If however, the noradrenaline content of the soluble supernatant is an artifact, it is surprising that several different laboratories, using different techniques which might be expected to lead to wide variations in quantitation, produce very similar estimates on the ratio of free/bound noradrenaline in peripheral sympathetic nerve endings (3, 4, 12, 13, 57, 58, 75, 100, 102, 103).

Histochemical studies also have been carried out to investigate

the possibility that a "free" pool of noradrenaline exists in sympathetic nerve endings. Such studies have shown a high concentration of noradrenaline in the terminal region of sympathetic nerves and only a small amount of transmitter in the connecting fibers (72). The transmitter present in the connecting fibers is concentrated at certain points which correspond to the presence of numerous storage granules. This evidence could be interpreted as implying that all the noradrenaline in the terminal regions of the nerve endings is stored within the granules, which are formed in the nerve cell body (25) and remain intact as they travel down the axon to the terminal. However, this interpretation does not take into consideration the distribution of the noradrenaline which has been released from the storage granules and also which has been taken up into the nerve ending again, after its release and subsequent action on the post-synaptic membrane.

This investigation is an attempt to obtain further biochemical information regarding the presence of unbound noradrenaline in sympathetic nerve endings, by examining changes induced in the monoamine oxidase activity of isolated perfused hearts, by infused noradrenaline (NA). The infused NA is assumed to be taken into the nerve tissues by the NA uptake system physiologically present in the nerve endings.

LITERATURE REVIEW

A. Distribution of Catecholamines Within Sympathetic Nerve Endings.

Three types of vesicles have been described in the axons of sympathetic neurons (47, 48, 95). These have been designated 'small agranular' vesicles with a diameter of 300-600 Å; 'small granular' vesicles with a diameter of 150-250 Å; and finally, 'large granular' vesicles, 600-1500 Å diameter with a central core 400-700 Å in diameter.

De Robertis (27, 28) in 1961, was the first to suggest that the small granular vesicles are associated with adrenergic neurons. Among the evidence which led to this suggestion are the observations that decreases in the amount of noradrenaline (NA) in denervated nicitating membranes of cats (98) and in transplanted dog hearts (76), parallel the disappearance of small granular vesicles from the nerves. Richardson (78) has shown that the nerves of the dilator muscle of the albino rabbit iris, which are predominantly adrenergic, have a large complement of small granular vesicles, while the cholinergic nerves to the sphincter muscle contain only small agranular vesicles.

Autoradiographical techniques (88, 105) have made indirect evidence available for the association of small granular vesicles with adrenergic nerves. By this technique it has been shown that exogenous tritiated NA is localized in the nerve terminals containing small granular vesicles.

A small proportion of agranular vesicles has been shown to be present in adrenergic nerves. It has been suggested that these contain acetylcholine, as they do in cholinergic nerves and that this is further

evidence for the Burn and Rand theory (9) of a cholinergic link in post-ganglionic sympathetic transmission. Contrary to this, it has been shown that the ratio of granular : agranular vesicles seen in preparations from cat iris (93), depend significantly on the method of fixation. This observation has led to the suggestion that the agranular or'empty' vesicles represent storage granules which have been depleted of the transmitter as a result of nerve stimulation, a drug effect or other causes.

The role of large granular vesicles in the storage of noradrenaline is, as yet, unclear. Generally, in sympathetic neurons they represent less than 5 per cent of the vesicle population (39, 44); further, their distribution is certainly not restricted to sympathetic neurons. These observations do not exclude the possibility that those large granular vesicles which are present in sympathetic neurons, do contain noradrenaline. Relevant to this possibility is the observation that the only granular vesicles present in the adrenergic nerves of some lower animals are of a size comparable to the large granular vesicles of higher animals (10).

The proportion of NA which is present in sympathetic nerves in a soluble form in the cytoplasm of the nerve terminal is still unclear. Hillarp (54) has put forward several pieces of evidence in favour of a pool of 'free' amine being present in the chromaffin cells under normal conditions, but did not produce any conclusive evidence as to its size. Calculations of NA per gram of whole tissue do not agree with the predicted concentration obtained from data from the vesicular fraction of the tissue. This discrepancy arises largely from the disruption of the vesicles during their separation. From the studies of Stjärne (85) it

has been suggested that 75 per cent of the total NA in a suspension of splenic nerve trunk vesicles is particle bound. However, this result is variable and von Euler (101) has suggested that as much as 80-90 per cent of the total NA content of splenic nerve is particle bound, the remaining small fraction being called 'transport NA', since he considered this fraction to be the one most readily available for mobilization and the pool through which NA must be transported during its release from the storage granules. Studies of the distribution of NA in brain homogenates show even greater variation with different experimental techniques (49), leading to the conclusion that much of the experimentally determined soluble NA content is an artifact. Similarly, measurements made in sympathetic ganglia have given rise to contradictory results (41, 73).

B. Theories of Release of Catecholamines from Sympathetic Nerve Endings.

Fatt and Katz (40) in 1952, studied the electrophysiological phenomena occurring in the neuromuscular junction of frog muscle fibres, with micro-electrodes. They and other workers (30, 31) observed, even during rest periods, minute transient fluctuations in voltage in the region of the end-plate, which they termed miniature end-plate potentials (mepp). After the discovery in 1955 by de Robertis (26), of synaptic vesicles a correlation was immediately made between nerve structure and electrophysiological behavior. The storage vesicles were considered to be in constant thermal agitation. Spontaneous release of transmitter substance was explained by assuming that bombardment of the terminal membrane by a small number of the vesicles, results in the joining of the vesicle and cellular membrane at complementary receptor sites, after which event, the contents of the vesicle are released into the synaptic cleft. Wherever adequate studies have been performed, release of transmitter from nerve endings has been shown to occur in quantal units, corresponding to the release of the contents of a finite number of storage vesicles (68). Unfortunately all the measurements have been made on cholinergic nerve-muscle junctions, cholinergic ganglionic synapses and other junctions involving glutamic acid as the transmitter (6, 38). Although miniature end-plate potentials can be recorded at adrenergic synapses (10, 11), there is very close electrical coupling between groups of smooth muscle fibres which so far has prevented the development of a suitable recording system. However, estimates of the quantal content of synaptic potentials at adrenergic synapses have been made (42, 86).

During electrical stimulation of sympathetic nerves, the size of the end-plate potential increases. This observation is explained by postulating that nerve stimulation increases the number of sites at which vesicles can react with the cellular membrane. This vesicle theory of release (26, 31), in which quantal release is equated with vesicle storage would appear logical in the absence of any further evidence.

Bass and Moore (5) have challenged the vesicle theory of release. They suggested that vesicles discharge their contents through axonal membranes whenever they acquire sufficient thermal energy to overcome those barriers which normally prohibit release of their contents. Axonal and vesicular membranes are charged (5), an observation which supports the suggestion that nerve impulses increase the release of transmitter by altering the electrical charge on either the vesicular or axonal membrane, or both (62). Further investigation has led to the rejection of this hypothesis, since it predicts a direct, simple relationship between transmitter release and temperature. Such a relationship has been shown in frog preparations within a definite temperature range (40), but investigations on the rat diaphragm-phrenic nerve preparation have shown a very complex relationship between these two parameters.

Most of the recent work on the theories of release of noradrenaline has been performed on adrenal medullary chromaffin granules for various technical reasons. Of these, the major advantages of this preparation are that the stimulant for release is acetylcholine, a chemical which is readily available in a pure form, is easily administered and antagonists to which are known; the anatomical arrangement of the adrenal medulla makes perfusion relatively easy and secretions can be recovered quant-

itatively; the secretory products are present in large amounts and it is generally agreed that the secretions consist of preformed materials rather than substances synthesised after release. The preparation is considered to be analogous to that of the vesicles in sympathetic nerve endings.

In the preceding section, the intracellular distribution of noradrenaline was examined, the conclusion being that the majority of the transmitter was stored in membrane-limited granules and the rest was 'free' in the cell sap. Blaschko and Welch (7) considered that the free amines were primarily involved during the release process. They suggested that the liberation of adrenaline from splenic nerves resulted from an increase in the permeability of the cellular membrane which consequently gave rise to loss of amine from the cytoplasm. In their scheme, these workers interpret the role of the granules as stores of catecholamines from which the cytoplasm is replenished. However, most evidence is in favour of the granules playing the primary role in release.

De Robertis and Vaz Ferreira (29) put forward some morphological evidence suggesting that catecholamine extrusion is the result of a process which they termed 'reverse pinocytosis', during which the catecholamine-containing granule becomes attached to the cell membrane and by coincident rupture of the granular and cellular membranes at the point of contact, the contents of the granule are released directly to the exterior of the cell.

Hillarp (54) subsequently proposed an alternative theory for the release of catecholamines from nerve terminals, based on the suggestion that the stimulus to secretion results in the granules giving

up their contents into the cell sap from which they escape to the cell exterior by diffusion through the cell membrane.

Finally,a mechanism proposed firstly by Cramer in 1928 (21) and more recently by Hagen and Barrnett (50), suggests that stimulation causes the whole granule, with its contents intact, to be extruded through the cell membrane. Electron microscopic evidence readily gives rise to a biassed result in favour of a direct 'extracellular' mechanism of release, with or without the granular membrane remaining within the cell. This arises due to the inability of the electron microscope to detect the movement of 'free' amines within the cell cytoplasm.

However, Carlsson and Hillarp (15) examined adrenal glands taken from rats after they had been subjected to several hours of medullary nervous stimulation. They found that the granular fraction was poor in both catecholamine and adenosine triphosphate (ATP), the latter having been suggested to form a storage complex with catecholamines in the granules (54). This evidence suggests that the granular catecholamines, rather than the free amines, are involved in the extrusion process, but it does not allow a further narrowing of the possible extrusion mechanisms, by differentiating between an involvement of the granules which gives rise to an increase in the intracellular free amine content followed by diffusion of the amine through the cell membrane, hereafter termed 'intracellular release', or alternatively to a mechanism whereby the granular contents are extruded to the exterior of the cell, following migration of the granules to the cell membrane. This latter mechanism would not give rise to any alteration in the free amine content of the cell and will be termed 'extracellular release'.

Further evidence which suggests the primary involvement of granular amines in the release process has been demonstrated by Schümann and Philippu (83). Calcium ions have been shown to be necessary for amine release from chromaffin cells; further to this, these workers have shown that the addition of calcium to a suspension or chromaffin granules, in vitro, accelerates the loss of catecholamines from the granules to the suspension medium. This evidence has been questioned since, on the grounds that high calcium concentrations are necessary to produce small effects and also because magnesium ions also have this facilitatory effect on in vitro preparations, but they strongly inhibit the release of catecholamines from intact chromaffin cells (37).

Douglas and his co-workers (33, 35) perfused cat adrenal glands with Locke's solution and evoked catecholamine secretion with acetylcholine and other compounds such as nicotine. They measured the concentrations of catecholamines and various adenosine nucleotides, including adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP), in the venous effluent and found that whenever catecholamines appeared in the venous effluent in response to a stimulus, large amounts of AMP and trace amounts of ADP and ATP were found. This evidence implied that the granular amines were immediately involved in the extrusion process because most of the nucleotides in the chromaffin cells are within the granules and little are free in the cytoplasm. Douglas also proposes that his evidence is in favour of extracellular release of catecholamines, since cell membranes are only very poorly permeable to adenosine nucleotides and thus a mechanism involving the release of the granular contents into the cell cytoplasm

followed by diffusion through the cell membrane, would not explain the observed results. Also, Douglas claims that it would be difficult to explain the relatively constant ratio of nucleotide to catecholamine which is consistently seen. One would have to assume that the various stimuli increased the chromaffin cell permeability to both 'free' amines and to nucleotides and that the permeability was increased to a parallel degree. Douglas and Poisner (33) supplied further evidence for the extracellular release of catecholamines from the medulla, by showing that stimulation of the medulla via the splanchnic nerve gave similar concentrations of catecholamines and nucleotides in the venous effluent as found in the perfusion experiments. Further, the ratio of the total amount of ATP and its metabolites (AMP and ADP) to the amount of catecholamines in the effluent (4.22 ± 0.7) was very close to the corresponding ratio found in the chromaffin granules of the cat adrenal medulla.

Douglas and Poisner (33) stimulated adrenal glands via the splanchnic nerve for short lengths of time (between 9 and 16 seconds) and measured the catecholamine and AMP content of successive drops emerging from the venous cannula after the beginning of stimulation. They showed that the concentrations of these compounds paralleled each other very closely, both reaching a peak within 10 seconds, after which time the output of both falls off. This is evidence against a secondary involvement of the granules, subsequent to a hypothetical escape of 'free' amines.

Theoretically, in order to lend support to an intracellular mechanism of release, it should be possible to show that an increase in the permeability of the chromaffin cell, such as brought about by acetylcholine, should allow the outward leakage of free amines. Evidence for

this theory is given support by the work of Douglas and Rubin (36) who suggest that calcium, in conditions known to increase the permeability of cell membranes, is a sufficient stimulus for catecholamine secretion.

The relationship of ATP hydrolysis to catecholamine release has been studied with the possibility in mind that ATP in the granules, is the critical link in catecholamine release. Hillarp (52) has reported ATPase activity in the chromaffin granule fraction and suggested that, when the chromaffin cell is stimulated, that the ATPase associated with the chromaffin granule, possibly in the membrane, is activated in a way which enables it to attack the ATP of the catecholamine storage complex, thus freeing the amines.

More recently, Douglas and Poisner (34) suggested that the presence of ATP metabolites in the venous effluent from the adrenal medulla might be attributable to the action of endothelial enzymes, since in control perfusions with ATP, little of the ATP survived passage through the adrenal vessels, most of it being converted to AMP and adenosine. When the hydrolysis of ATP was reduced by removing calcium and magnesium ions and by adding 1 - 2 mM EDTA, 80 per cent of the ATP passed through the gland unmetabolized. Barium was used to evoke secretion in this instance. The ratio of catecholamine to ATP was 11:1, with most of the ATP intact. The comparable ratio found in experiments on the intracellular measurement, was greater than 100:1, with the main nucleotide being AMP. The conclusion drawn from this was that the hydrolysis of intragranular ATP is not a critical step in the release process. These observations make it difficult to explain any intracellular mechanism of release of catecholamines since it is unlikely that if catecholamines and

ATP are liberated from the granules into the cell sap, that ATP should, on the one hand, escape hydrolysis by intracellular enzymes and, on the other hand, should penetrate the chromaffin granules so readily that the catecholamine:ATP ratio is maintained at approximately the same value as in the storage granule.

Douglas (32) concludes from these observations that catecholamines are extruded from the chromaffin cells and, by analogy, from the sympathetic nerves, by an extracellular mechanism. Three possibilities exist: extrusion of the whole granule; extrusion of the granular contents by reverse micropinocytosis or by a process involving the transient fusion of the granular and cellular membranes, resulting in a great increase in permeability at the point of fusion, which allows diffusion of the granular contents out of the cell.

In order to distinguish between these possibilities, the contents of the effluent from stimulated preparations has been examined. Carlsson and Hillarp (15) measured the protein content of granule fractions isolated from adrenal glands which had been stimulated via the splanchnic nerve for several hours. They found that the protein content was not lowered to the same extent as the catecholamine and ATP content in this fraction, as compared with control values. From this they concluded that either the granules had given up their catecholamine and ATP while retaining protein, or that new granular protein had formed during prolonged stimulation.

Poisner, Trifaró and Douglas (74, 97) measured the efflux of phospholipids and cholesterol, which are the principal lipids of the chromaffin granule membrane, and found that it rises little, if at

all, on stimulation of the chromaffin cells. Correspondingly, these workers found no fall in the phospholipid and cholesterol contents of a subcellular fraction containing chromaffin granules, following medullary stimulation.

Electron microscopic work has shown that after stimulation the subcellular fraction containing chromaffin granules is rich in profiles with the appearance of empty granules (66). Further work which suggests that the granular membrane remains within the cell after stimulation, has been performed by Viveros (99). After the observation that a portion of the activity of dopamine-β-hydroxylase in the adrenal gland is associated with the vesicle membrane, Viveros used this enzyme, which is involved in the conversion of dopamine to noradrenaline as a marker to follow the fate of the adrenal storage vesicles after the release of noradrenaline from them. He used three groups of rabbits: a control group; a group whose adrenal glands were stimulated to secrete by insulin-induced hypoglycaemia and a group treated with reserpine to deplete the adrenal glands of their catecholamines. The two drugs were used since they produce different responses in the adrenal medulla. Reserpine blocks the uptake of catecholamines into storage vesicles (16), resulting in the inability of the adrenal glands to maintain their catecholamine stores. Insulin causes hypoglycaemia which induces reflex neurogenic stimulation of the adrenal gland that results in the secretion of adrenaline and noradrenaline. Viveros, et al. showed that the dopamine-β-hydroxylase content of the insulin-stimulated glands was less than that of the control glands, suggesting that the enzyme is released in vivo as well as from isolated perfused glands. Low doses of reserpine (1mg/kg)

did not change the dopamine-β-hydroxylase during the catecholamine depletion phase. The adrenal glands were homogenized and centrifuged to separate the supernatant from the particulate fraction containing the storage granules. Generally, in the reserpine-treated rabbits, the distribution of dopamine-β-hydroxylase and catecholamines between the particulate fraction and the supernatant did not differ from that of the controls.

This evidence has led to the conclusion that neurogenic secretion is characterized by release of the soluble contents of the granular storage vesicles to the exterior of the cells, while the storage vesicle membranes remain within the cell.

Controversial evidence has been submitted concerning the fate of the storage vesicle membranes, after they have released their contents. Electron microscopic studies have shown images resembling empty vesicles within the cell (66). However, de Robertis' theory of reverse pinocytosis implies that the granular membrane is incorporated into the cellular membrane. If this latter mechanism is a true one, stimulation of the chromaffin cells should lead to a decrease in the lipid content of the subcellular fraction which contains the chromaffin granules,and images resembling emptied granules should not be seen in the electron microscope. Evidence already quoted is contrary to this hypothesis and therefore the conclusion to be drawn is that, after releasing their contents to the cell exterior, either by a definite rupture of the adherent cellular and granular membranes; or by a greatly increased permeability of the membranes at the site of adhesion, the emptied granules remain within the cell, at least for a certain finite time.

At the present time, evidence is overwhelmingly in favour of an extracellular method of release of noradrenaline from adrenal medulla cells and therefore, very probably, from sympathetic nerve endings. However, this bias has been established partly through the lack of appropriate methodology for the measurement of the free, fluctuating pool of noradrenaline within the cell. Further evidence concerning the size of this pool with respect to various procedures would be beneficial in the establishment of the method or methods of release of noradrenaline from sympathetic nerve endings.

C. Localization of Monoamine Oxidase with respect to the Sympathetic Nerve Endings.

Studies by Kopin and Axelrod (60) have emphasized the role of monoamine oxidase (MAO) in the function of the sympathetic nervous system. The activity of MAO is thought to be responsible for the breakdown of noradrenaline which is released within the sympathetic nerve endings preceding its catabolism to inactive metabolites. This role assumes that MAO is localized within the nerve ending. Snyder, Fischer and Axelrod (84) studied the pineal gland of the rat, of which a large proportion of the total mass is composed of sympathetic nerve endings. They found that sympathetic denervation of the gland causes a 50 per cent reduction in the MAO activity of the organ. A 28 per cent reduction in MAO activity of the rat submaxillary gland was seen after sympathetic denervation (84). This evidence suggests that at least a considerable amount of the total MAO activity of these tissues is located within the sympathetic nerves. Further evidence in support of MAO being present within the cells of the adrenergic nervous system is given by Consolo, et al. (18) who found that MAO is selectively present in the adrenergic cells of sympathetic ganglia while being absent in isolated cholinergic or sensory cells. Giacobini, et al. (45) studied the developmental changes of MAO in chick embryo spinal and sympathetic ganglia and found that the ratio of MAO activity between sympathetic and spinal ganglia is almost 5:1 at the time of hatching, an observation which is in agreement with the prominent role known to be played by MAO in sympathetic nervous activity.

In her work on the accumulation of MAO in rat sciatic nerves after constriction, Dahlström (22) measured an increase in MAO activity in the proximal part of the nerve, to an extent of 240 per cent the normal level after constricture for seven days. She found also, that sympathectomy did not significantly reduce the MAO activity in unligated nerves in comparison with normal values but that in ligated nerves the MAO activity at 7 days was significantly higher in normal nerves than in sympathectomized tissues. Dahlstrom concluded from her study that MAO accumulates proximal to a constriction of the sciatic nerve and that at least a significant part of this increase is due to a relatively fast moving MAO fraction in the sympathetic adrenergic axons in this nerve.

Roth and Stjärne (80) suggested that MAO may be present in the amine storage granules within adrenergic nerve endings as well as in the mitochondria of the nerves (79). More recently de Champlain, et al. (24) suggested also that MAO may be associated with catecholamine storage granules. Schnaitman, et al. (81) have been able to separate the outer and inner membranes of rat liver mitochondria and have been able to establish that MAO is located on the outer of these two membranes.

D. Chemical Sympathectomy with 6-Hydroxydopamine.

Some of the studies reported in this thesis involve the use of 6-hydroxydopamine to cause chemical sympathectomy. Biological research has frequently used the techniques of removal or destruction of organs in order to study their physiological role. Cannon and Rosenblueth (14), and also Trendelenburg (96) used applications of the method in their studies on the sympathetic nervous system. In situations in which the organ to be studied is innervated by a single nerve, or several discrete nerves which are easily accessible, the method of choice for denervation of these organs is surgical denervation. This applies to the nictitating membrane, iris and salivary gland. However, other organs have a much more diffuse nervous supply and different methods must be adopted. Immunosympathectomy was introduced and used by various workers including Levi-Montalcini and Booker (64), Cohen (17), and Levi-Montalcini and Angeletti (63). The procedure destroys or even prevents the development and differentiation of a great part of the pre- and paravertebral sympathetic ganglia. It involves the administration of an antibody against a protein essential for the development of sympathetic and sensory ganglia to newborn animals of various species.

More recently a simpler technique has been developed in which adrenergic nerve endings are selectively destroyed by 6-hydroxydopamine (6-OHDA) (93, 95). This development was initiated by the observation that 5-hydroxydopamine (5-OHDA) and its β-hydroxylated and o-methylated metabolites are stored in sympathetic nerve endings and released as false transmitters by electrical nerve stimulation (94). 5-OHDA was administered to rats and cats in doses which reduced the noradrenaline content

of peripheral sympathetically innervated organs to less than 10 per cent of control values (90). At this dose level, the vesicles of the adrenergic nerves were filled with dense osmiophilic material, whereas the vesicles of cholinergic nerves did not change in their appearance (94). The administration of similar doses of 6-OHDA had a different effect. After two or three days, electron microscopy showed that the adrenergic nerves were in various stages of degeneration, while smooth muscle cells, Schwann cells and cholinergic nerves were unaffected. The cell bodies of sympathetic ganglia are seen to be unaffected by 6-OHDA, an observation which supports the theory that it is the nerve endings alone which are destroyed by the drug (95). Thoenen and Tranzer (91) have measured noradrenaline levels of the chemically sympathectomized organs and have found a long lasting depletion of the transmitter which corresponds to the morphological evidence for the destruction of the adrenergic nerve terminals. Similarly, cardiac tyrosine hydroxylase activity is significantly reduced (69) by 6-OHDA, which adds support to the view that this enzyme is located within adrenergic neurons. However, a concurrent two-fold increase in adrenal tyrosine hydroxylase activity is seen. Thoenen and Tranzer (91) suggest from their evidence, that the long-lasting noradrenaline depletion represents a reliable measure of the destruction of the sympathetic nerve terminals.

The precise mechanism of action of 6-OHDA in the destruction of adrenergic nerve terminals is still uncertain (89). It has been shown that a critical dose of 6-OHDA is necessary to produce the long-lasting noradrenaline depletion (91) and it is assumed that nerve ending destruction depends on the uptake of a critical amount into the nerve terminal.

Low doses of 6-OHDA can be taken up and stored in sympathetic nerve endings and can be released as a false adrenergic transmitter, but higher doses lead to the destruction of the adrenergic nerve terminals and therefore of their own storage sites. Reserpine pretreatment did not prevent either the morphological changes which take place in the nerve endings following 6-OHDA administration, or the reduction in tyrosine hydroxylase activity, thus showing that storage of 6-OHDA is not a prerequisite for its destructive action (92).

The method of chemical sympathectomy compares favourably with those of surgical and immunological procedures and is frequently preferred mainly because of its simplicity (18, 19, 51, 59). One disadvantage which the method shares with immunosympathectomy is the organ variation which occurs, probably due to differences in the density of the adrenergic innervation and also on the blood supply which carries the drug to its site of action.

With respect to the mechanism of action of 6-OHDA, in addition to the observation that the drug is efficiently accumulated within the adrenergic nerve endings (67, 87), a second property of the drug which seems significant, is its extreme susceptibility to non-enzymatic oxidation. The formation of hydrogen peroxide from 6-OHDA has been shown in vitro and from this it could be assumed that tissue damage could be caused by this product at the sites of its highest concentration, which are the adrenergic nerve endings. Alternatively, the nerve endings might be destroyed by the covalent binding of the drug to some tissue component.

A STATEMENT OF THE PROBLEM

Although it appears logical that a pool of free NA exists in the nerve endings through which NA taken into the nerve ending passes before becoming bound in the storage granules, and although differential centrifugation indicates that a substantial amount of NA is found in the high speed supernatent which can be increased by perfusion of an organ with NA (46), no methods have evolved to estimate the size of that postulated pool or to observe changes in its concentration of NA in nerves, in situ. This project is an attempt to use an enzyme assay method for MAO as an indication of changes in concentration of free NA in that postulated pool in the sympathetic nerve endings of the isolated guinea-pig heart.

Fig. 1, a diagrammatic representation of a sympathetic nerve ending, illustrates the reasoning behind the proposed method of detecting changes in the concentration of free NA in the nerve ending. The method is based on the assumption that when kynuramine, a synthetic substrate for MAO, is added to the system, it will compete with NA for a position on the enzyme. Kynuramine is oxidized by MAO to a fluorescent product, 4-hydroxyquinoline (4-OHQ) (104), which can be determined fluorometrically by the method of Krajl (61) for the measurement of MAO activity. If the concentration of intracellular NA rises it will more effectively compete with the kynuramine and cause a fall in the output of 4-OHQ. Changes in intracellular free NA will cause reciprocal changes in the output of 4-OHQ.

A comparison between noradrenaline and kynuramine, competitive substrates for MAO and their respective products is presented below:

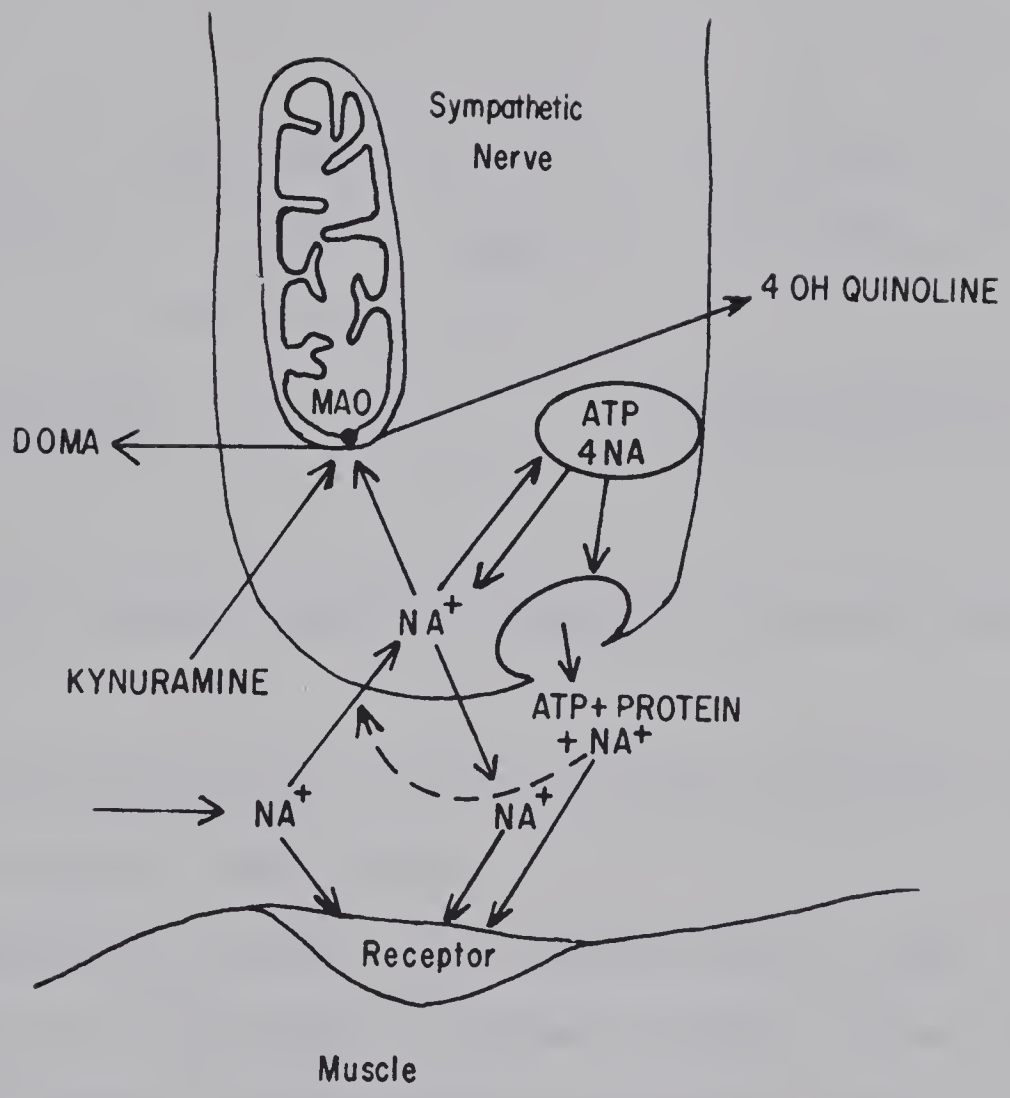
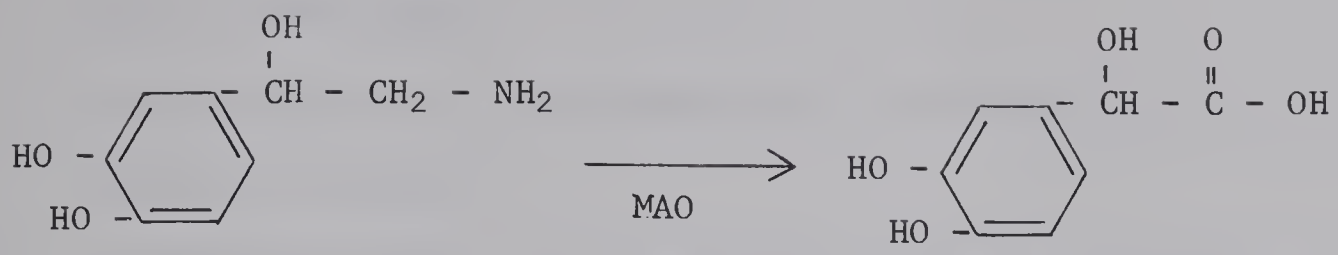
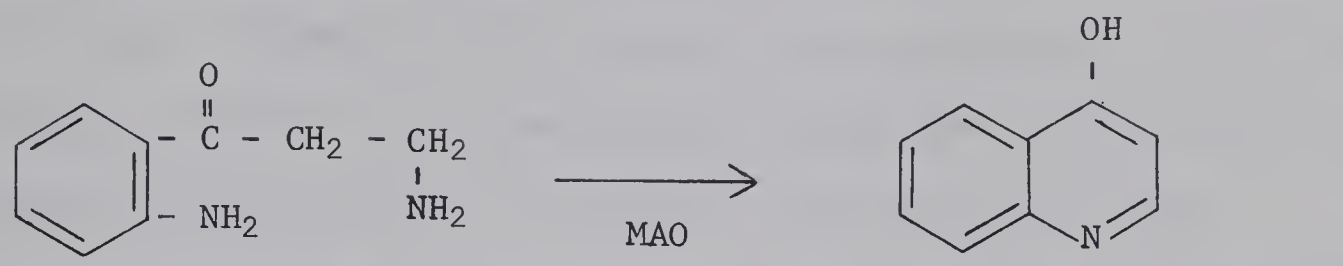


FIG. 1. Diagrammatical representation of a sympathetic nerve ending, showing the relationships between monoamine oxidase (MAO), two of its substrates, noradrenaline (NA) and kynuramine and their metabolic products, dihydroxymandelic acid (DOMA) and 4-hydroxyquinoline.



NORADRENALINE (NA)

DIHYDROXYMANDELIC ACID (DOMA)



KYNURAMINE

4-HYDROXYQUINOLINE (4-OHQ)

The problems involved in testing the proposed method are the following:

1. To demonstrate,in vitro, that NA acts as a competitive inhibitor of the kynuramine - MAO reaction.
2. To demonstrate, in vivo, that a steady state of 4-OHQ output can be obtained when kynuramine is perfused through a tissue containing adrenergic nerves and that infusions of NA will cause an inhibition of that reaction, presumably due to uptake of NA into the nerve ending.
3. To demonstrate that a block of NA uptake, by DMI treatment, can prevent the inhibitory action of infused NA and prevent the fall in 4-OHQ output.
4. To demonstrate that a drug which presumably causes an intracellular release of NA (reserpine), can cause an in situ inhibition of MAO

and of 4-OHQ output.

5. To demonstrate that the observed fall in 4-OHQ output is not due to vasomotor activity.
6. To demonstrate that a good substrate for MAO and therefore a similar competitor to noradrenaline, but which is less well taken up into nerve endings, is less effective as an inhibitor.
7. To present evidence that MAO located within the nerves is the chief enzyme involved in this reaction and against the postulate that the effects observed are due to reaction with extraneuronal MAO.

The following report attempts to deal with each of these necessary requirements for testing the method and to draw conclusions regarding the reliability of the method as an indication of changes in the concentration of free NA in sympathetic nerve endings.

METHODS

A. Heart Perfusion Studies.

The perfusion apparatus was the same as that used by Nash, Wolff and Ferguson (71) and is illustrated in Fig. 2. Two hearts were perfused simultaneously.

Male guinea-pigs weighing between 250-300 g were killed by a blow on the head; the hearts were removed, cannulated via the aorta and perfused by a modified Langendorff method at a constant flow rate of 6 mls/min. The perfusion fluid was a Krebs-bicarbonate medium (see Appendix I), aerated with a 95% oxygen, 5% carbon dioxide gas mixture and warmed to 37^{o}C by passage through a heating coil. The perfusion fluid was delivered to the hearts by a peristaltic perfusion pump (Harvard Instrument Company) and samples of the effluent were collected in 50 ml tubes, taken at 2 minute intervals. To prevent any continued activity after efflux, 1 ml of a 10 per cent solution of trichloroacetic acid (TCA), was added to each tube. Noradrenaline was introduced into this perfusion fluid from an infusion pump, entering the system just above the hearts. In this way a very small dead space was achieved. Other variations in the perfusion fluids were made by addition to the Krebs-bicarbonate medium, before it passed through the peristaltic pump and heating coil.

The ionic composition of the Krebs-bicarbonate medium is given in Appendix I, together with details of the concentrations of all other solutions used in the perfusion experiments.

The perfusion experiments were performed on a similar time schedule so that the effects of different agents might easily be compared.

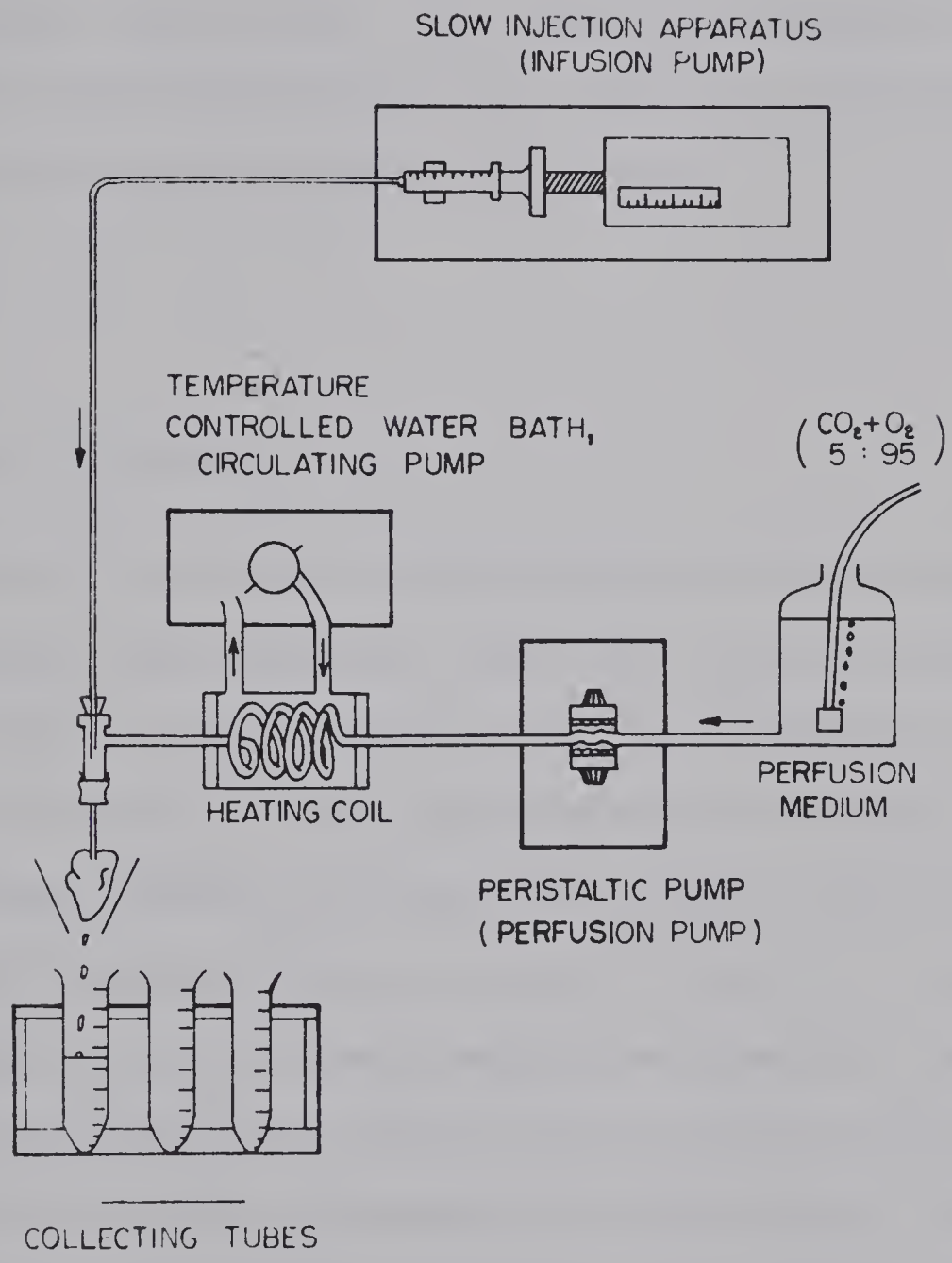


FIG. 2. Diagram of the perfusion apparatus.

The hearts were perfused for 40 minutes with Krebs-bicarbonate medium containing kynuramine (188μM). After twenty minutes' perfusion to establish an equilibrium with a steady state output of 4-hydroxyquinoline, test conditions were applied for a 10 minute period followed by a 10 minute period for recovery from the test. The schedule was completed with an 8 minute perfusion with Krebs solution only. Details of each perfusion sequence are given in the chapter reporting the results.

B. Electrical Stimulation.

To test the effect of nerve stimulation on the output of 4-OHQ during perfusion with kynuramine, rabbit hearts were stimulated electrically in the region of the sino-atrial node, by an adaptation of the method of Amory and West (1). The stimulation was at a strength of 20 volts; duration 1 msec; frequency 250/second for 5 seconds. This stimulation was repeated 3 times at 1 minute intervals followed by an 8 minute rest period. Typically this 11 minute sequence was repeated 3 times in the course of an experiment. The rate and force of contraction of the hearts was measured by a pressure transducer and recorded using a Beckman polygraph. Samples of the effluent were collected at 2 minute intervals and assayed for changes in 4-OHQ output as an indication of changes in MAO activity and the intracellular concentration of NA.

C. Measurement of Monoamine Oxidase (MAO) Activity.

The method is the fluorometric adaptation by Krajl (61) of the original procedure of Weissbach (104) for the determination of MAO activity.

(a) In vivo

Samples were collected from the perfusion of guinea-pig hearts as previously described. Any protein in the samples was precipitated by TCA and centrifuged if necessary. One ml aliquots from each sample were pipetted into tubes containing 2 mls of 1 N sodium hydroxide (NaOH), to develop the fluorescence. After thorough mixing, the fluorescence of the solutions was measured in an Aminco-Bowman Spectrofluorometer, at an activation wavelength of 315 nm and a fluorescent wavelength of 380 nm.

(b) In vitro

In vitro studies on MAO were carried out using an enzyme preparation from guinea-pig livers as the source of MAO. The method of Schneider and Hogeboom (82) was used to isolate the mitochondria and therefore the MAO, from the liver tissue.

Liver tissue was excised from male guinea-pigs which had been killed by a blow on the head. The tissue was weighed and homogenized in 9 volumes of 10 per cent sucrose. The homogenate was centrifuged at 1000 rpm for 10 minutes in an International centrifuge to remove the cell debris. The supernatant was pooled and then centrifuged at 9200 g for 15 minutes in a Lourdes refrigerated centrifuge. After discarding the supernatant, the mitochondrial pellets were solubilized with 4.5 mls Triton X, diluted 1 in 1000. The contents of all the tubes were pooled, re-homogenized in a Potter homogenizer and the resulting solution was used as the source

of MAO in in vitro tests. The procedure was carried out using refrigerated solutions and the tissue and homogenates were kept on ice whenever possible.

Incubation tubes were set up to investigate the in vitro inhibitory effects of various agents on the oxidative deamination of kynuramine to 4-hydroxyquinoline. The details of the tube contents are set out in Appendix I. The tubes were incubated, with shaking, for 30 minutes at 37 ^{o}C, when the enzymatic reaction was stopped by the addition of 2 mls of 10 per cent TCA. The precipitated proteins were centrifuged down; 1 ml of the supernatant was added to 2 mls of 1 N NaOH and the fluorescence of the solution was measured as described above. Double reciprocal plots (65) of the data were drawn to investigate the inhibitory effects of the various agents on the conversion of kynuramine to 4-hydroxyquinoline.

D. Catecholamine Assay.

In the experiments in which 6-hydroxydopamine was used to effect chemical sympathectomy, part of the heart tissue was taken and assayed for noradrenaline. When the NA content of the treated hearts was compared with the control values, a measure of the effectiveness of the sympathectomy was obtained.

After removal of the hearts, either directly from the animal or from the perfusion apparatus, they were cut open and blotted to remove blood or perfusion fluid, frozen with liquid nitrogen and pulverized, individually, in a metal di-press, which was also cooled with liquid nitrogen. The fine frozen powder which resulted was mixed uniformly and transferred into a second, smaller di-press and pelleted. In this way a uniform dis-

tribution of atrial and ventricular tissue in the pellet was achieved. Two-thirds of the pellet was taken for noradrenaline assay, while the remainder was assayed for MAO activity.

(a) Procedure {from Anton and Sayre (2)}.

The entire procedure was carried out with cold reagents and glassware. Details of the reagents are given in Appendix I, as is the procedure for the activation of the aluminium oxide. The tissue was weighed, homogenized in four times its weight of 0.4N perchloric acid and centrifuged at 1000 rpm for 10 minutes to remove the cell debris. Two mls of the supernatant were added to 2g of activated aluminium oxide. With constant mixing on a magnetic stirrer 7 mls of Tris buffer (pH 9) were added to the mixture, while the pH was adjusted to 8.4 with 1 N NaOH or hydrochloric acid (HCl). The mixture was transferred to a centrifuge tube after stirring for 3 minutes. The residue was allowed to settle and the supernatant removed by suction. Each sample was washed with 5 mls of water, stirred, centrifuged and the supernatant discarded. The catecholamines were eluted by adding 3 mls 0.05 N perchloric acid and shaking for 15 minutes. To 1 ml aliquots of each sample, 1 ml 0.1 M EDTA (ethylenediaminetetraacetic acid) solution and 0.2 mls 0.1 M iodine solution were added. Two minutes later 0.2 mls alkaline sulphite were added followed, 2 minutes later, by 0.2 mls 5 N acetic acid. The tubes were placed in a boiling water bath for 3 minutes and then cooled to room temperature. The fluorescence of the solution was read on the fluorometer at an activation wavelength of 385 nm and a fluorescent wavelength of 485 nm. This reading gave a measure of the noradrenaline content of the tissue.

(b) Standards and blanks

In order to calculate the percentage recovery achieved during the procedure, internal standards (IS_A and IS_B) were processed as follows:

(i) IS_A - 1 ml of tissue supernatant + 1 ml NA standard

(ii) IS_B - 1 ml of tissue supernatant + 1 ml 0.05N $HClO_4$

Other standards used in the procedure were:

(i) Reagent blank - 1 ml 0.05N $HClO_4$, developed the same way as the tissue extracts.

(ii) Noradrenaline standard - 1 ml 0.5 µg/ml NA in 0.05N $HClO_4$, developed the same way as the tissue extracts.

(iii) Tissue blank - 1 ml supernatant of each type of tissue, developed the same way as tissue extracts except that the order of addition of iodine and alkaline sulphite reagents was reversed so that all reagents were present but no oxidation took place.

(c) Calculation of NA content of tissue

$$\text{Dilution factor} = \frac{(\text{weight of tissue} + \text{vol. of } HClO_4)}{\text{weight of tissue}} \times 3/2$$

$$\text{Percentage recovery} = \frac{(IS_A - IS_B) \times 100}{NA_{STD}}$$

(i) Multiply the meter reading by the multiplier value to give the relative fluorescence index (RFI).

(ii) Corrected RFI = RFI for NA standard - RFI for reagent blank for NA_{STD}.

(iii) Similarly, corrected RFI = RFI for tissue - RFI for reagent blank for tissues.

(iv) μg of NA/g of tissue

$$= \frac{\text{Corrected RFI}_{(\text{tissue})} \text{ X Dilution factor X } 0.5}{\text{Corrected RFI}_{(NA_{STD})}}$$

E. Chemical Sympathectomy.

Guinea-pigs were treated with 6-hydroxydopamine (100 mg/kg I.P.) on day 1 and again on day 3 of the experiment. On day 10 the animals were killed by a blow on the head and the brain, heart and vas deferens of each animal were removed. The hearts of some of the animals were used in in vivo perfusion studies in a similar manner as described earlier. The remaining hearts and the brains and vas deferens from all the animals, were divided for NA and MAO assays.

RESULTS

A. In Vitro Studies on Monoamine Oxidase Activity.

1. The effect of noradrenaline and reserpine on the oxidative deamination of kynuramine to 4-hydroxyquinoline by monoamine oxidase.

In a suitably buffered medium, kynuramine is converted to a fluorescent product, 4-hydroxyquinoline under the influence of monoamine oxidase. In order to test if any in vivo effect of either noradrenaline or reserpine on this conversion is due to a direct inhibitory effect on the enzyme, increasing amounts of these two agents were added, in separate experiments, to the in vitro MAO assay system.

Figure 3 shows that NA at concentrations of 30 μM and greater, inhibits the formation of 4-hydroxyquinoline. The double reciprocal plot of the Lineweaver-Burke type indicates that this inhibition is probably competitive.

Figure 4 shows the results of a similar test with reserpine. Inhibition of the conversion of kynuramine to 4-hydroxyquinoline occurs only at concentrations of reserpine greater than 100 μM. That this inhibition was likely to be of a competitive type was indicated by the double reciprocal plot.

2. The effect of desmethylimipramine on the conversion of kynuramine to 4-hydroxyquinoline.

Table I shows that desmethylimipramine has no inhibitory effect on the oxidative deamination of kynuramine.

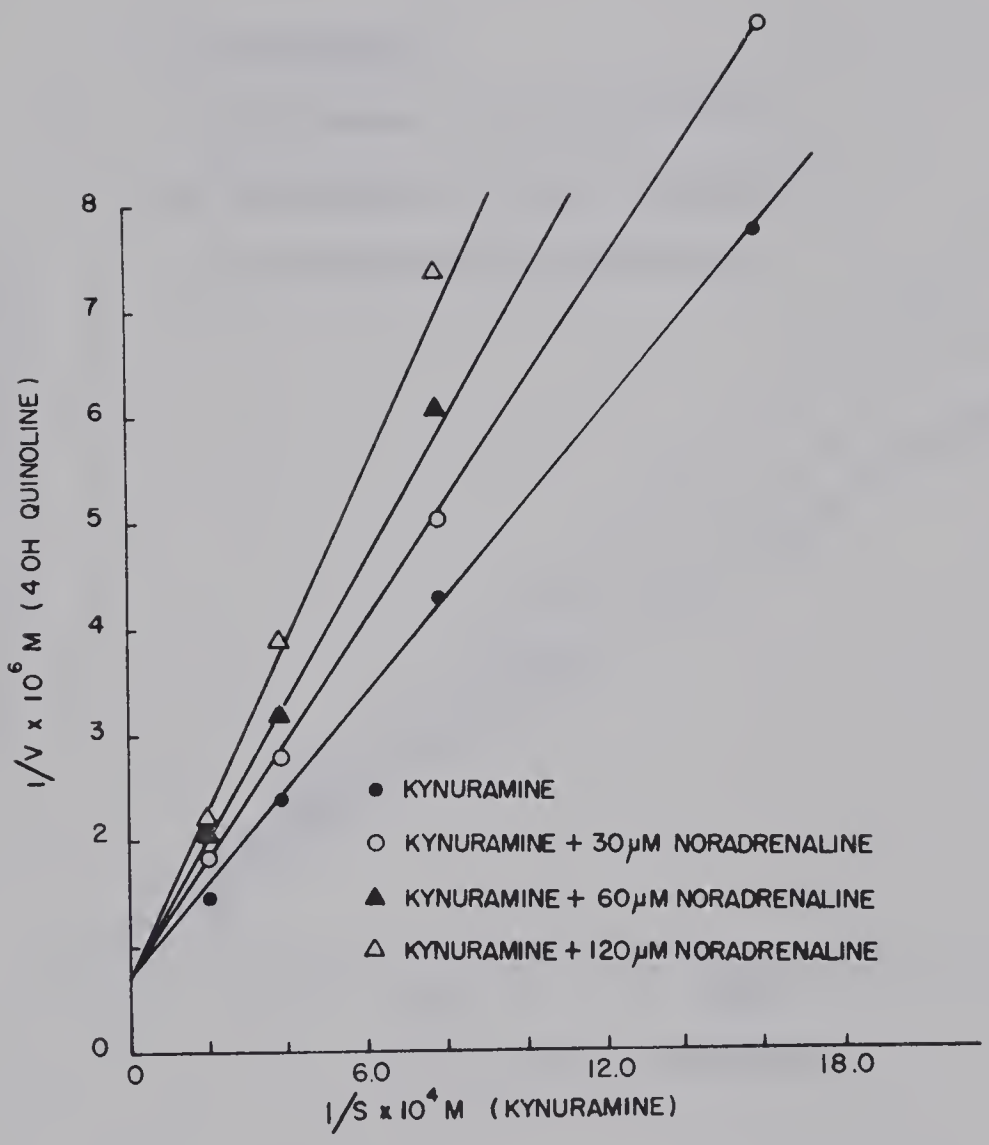


FIG. 3. Double reciprocal plot showing the inhibitory effect of noradrenaline on the formation of 4-hydroxyquinoline at NA concentrations greater than 30 μM. The source of the MAO was guinea-pig liver tissue.

Each value is the mean of 4 determinations.

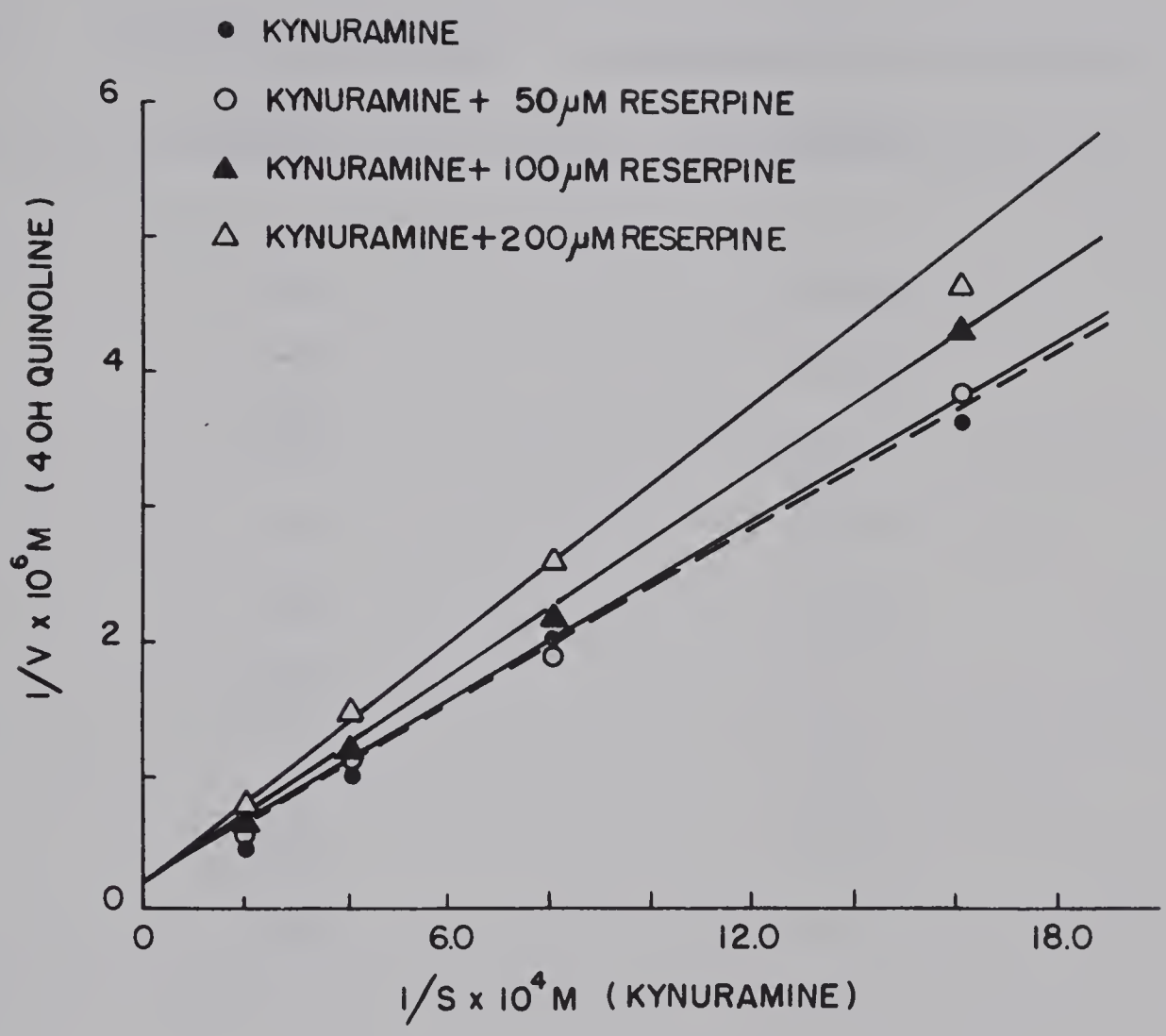


FIG. 4. Double reciprocal plot showing the inhibitory effect of reserpine on the formation of 4-hydroxyquinoline at reserpine concentrations greater than 100 μM. The source of the MAO was guinea-pig liver tissue.

Each value is the mean of 4 determinations.

TABLE I. Influence of desmethylimipramine on the oxidation of kynuramine to 4-hydroxyquinoline by monoamine oxidase.

KYNURAMINE (µg/ml)	DESMETHYLIMIPRAMINE (µg/ml)	4-HYDROXYQUINOLINE PRODUCED (µg/ml)
2.08	0	0.42
2.08	20	0.42
2.08	200	0.42
4.15	0	0.69
4.15	20	0.78
4.15	200	0.74
8.30	0	1.47
8.30	20	1.47
8.30	200	1.47

Each value is the mean of 2 determinations.

3. The effect of 6-hydroxydopamine pretreatment on the level of MAO activity in guinea-pig and rat heart, brain and vas deferens.

Table II shows that the level of MAO activity in the hearts and vas deferens taken from guinea-pigs sacrificed 10 days after the first of two injections of 6-OHDA (100 mg/kg I.P.), was reduced more than 50 per cent in comparison with control values. The level of activity in the brain tissue was not affected by 6-OHDA. Rat tissues showed similar reductions in the level of activity as seen in the guinea-pig.

4. The effect of 6-hydroxydopamine pretreatment on the level of noradrenaline in guinea-pig and rat heart, brain and vas deferens.

Table III shows that the 6-OHDA pretreatment reduces the noradrenaline content of both guinea-pig and rat hearts and vas deferens between 60 and 80 per cent, while the brain levels are not affected.

B. In Vivo Studies on Monoamine Oxidase Activity.

1. Prolonged infusion of guinea-pig hearts with kynuramine.

Figure 5 shows that infusion of 188 μM kynuramine into guinea-pig hearts over a prolonged period, causes maximum output of 4-OHQ to be obtained within 10 minutes. This level of output declined slowly over the duration of the infusion, which was 1 hour.

TABLE II. The effect of 6-hydroxydopamine pretreatment on the monoamine oxidase activity in rat and guinea-pig tissues.

TISSUE	TREATMENT	RESULT (EAU) ± S.E.	% REDUCTION	'P'
		GUINEA PIG		
Heart	Control	1.35 ± 0.335		
	6-OHDA	0.52 ± 0.102	61.5	0.025
Vas Deferens	Control	1.02 ± 0.104		
	6-OHDA	0.35 ± 0.120	65.7	< 0.005
Brain	Control	1.87 ± 0.094		
	6-OHDA	1.77 ± 0.063	5.4	N.S.*
		RAT		
Heart	Control	0.84 ± 0.063		
	6-OHDA	0.33 ± 0.030	60.6	< 0.005
Vas Deferens	Control	0.89 ± 0.038		
	6-OHDA	0.22 ± 0.022	77.0	< 0.005
Brain	Control	1.27 ± 0.110		
	6-OHDA	1.16 ± 0.112	8.7	N.S.**

6-OHDA Treatment: 2 doses, 100 mg/Kg I.P. on days 1 and 3.
Tissues used on day 10.

EAU (Enzyme Activity Units): Fluorescence/g tissue.

S.E.: Standard error.

P: Probability (Student t-test).

N.S.: Not significant. * P = 0.200
** P = 0.250

Complete data presented in Appendix II, Tables IIA and IIB.

TABLE III. The effect of 6-hydroxydopamine pretreatment on the noradrenaline content of rat and guinea-pig tissues.

TISSUE	TREATMENT	NA (µg/g) ± S.E.	% REDUCTION	'P'
		GUINEA-PIG		
Heart	Control	0.91 ± 0.132		
	6-OHDA	0.29 ± 0.042	68.1	< 0.005
Vas Deferens	Control	2.22 ± 0.486		
	6-OHDA	0.50 ± 0.115	77.5	< 0.005
Brain	Control	0.22 ± 0.036		
	6-OHDA	0.21 ± 0.025	4.5	N.S.*
		RAT		
Heart	Control	0.48 ± 0.070		
	6-OHDA	0.10 ± 0.044	79.8	< 0.01
Vas Deferens	Control	1.34 ± 0.437		
	6-OHDA	0.61 ± 0.062	54.5	0.15
Brain	Control	0.34 ± 0.038		
	6-OHDA	0.41 ± 0.031	0	N.S.

* P > 0.350

Complete data presented in Appendix II, Tables IIIA and IIIB.

2. The effect of simultaneous infusions of kynuramine and noradrenaline on the output of 4-hydroxyquinoline.

Guinea-pig hearts were perfused for 40 minutes with Krebs-bicarbonate solution containing 188 μM of kynuramine. After 20 minutes had elapsed to insure that a maximum plateau level of 4-OHQ output had been attained, sufficient noradrenaline was infused from a motor driven syringe into the flowing perfusion fluid to ensure a concentration of 2 μM of NA was perfused simultaneously with the kynuramine for the following 10 minutes. After that time, kynuramine infusion was continued for a further 10 minutes followed by a recovery period of 8 minutes, when the hearts were perfused with normal Krebs-bicarbonate medium which concluded the experiment. Figure 5 shows that infusion of noradrenaline caused a sharp fall in the production of 4-OHQ, which lasted for the duration of the infusion. After the NA infusion was turned off, the 4-OHQ production returned to its previous maximum level.

3. The effect of desmethylimipramine (DMI) on the 4-OHQ production during kynuramine infusion and during the simultaneous infusions of kynuramine and noradrenaline.

Desmethylimipramine affects sympathetic nerve endings by preventing the reuptake of noradrenaline into the nerve terminal after it has been released as a result of nerve stimulation, or by other means (4). If MAO is mainly confined to the sympathetic nerves, DMI should prevent the uptake of infused NA and thus eliminate the competitive effect between NA and kynuramine.

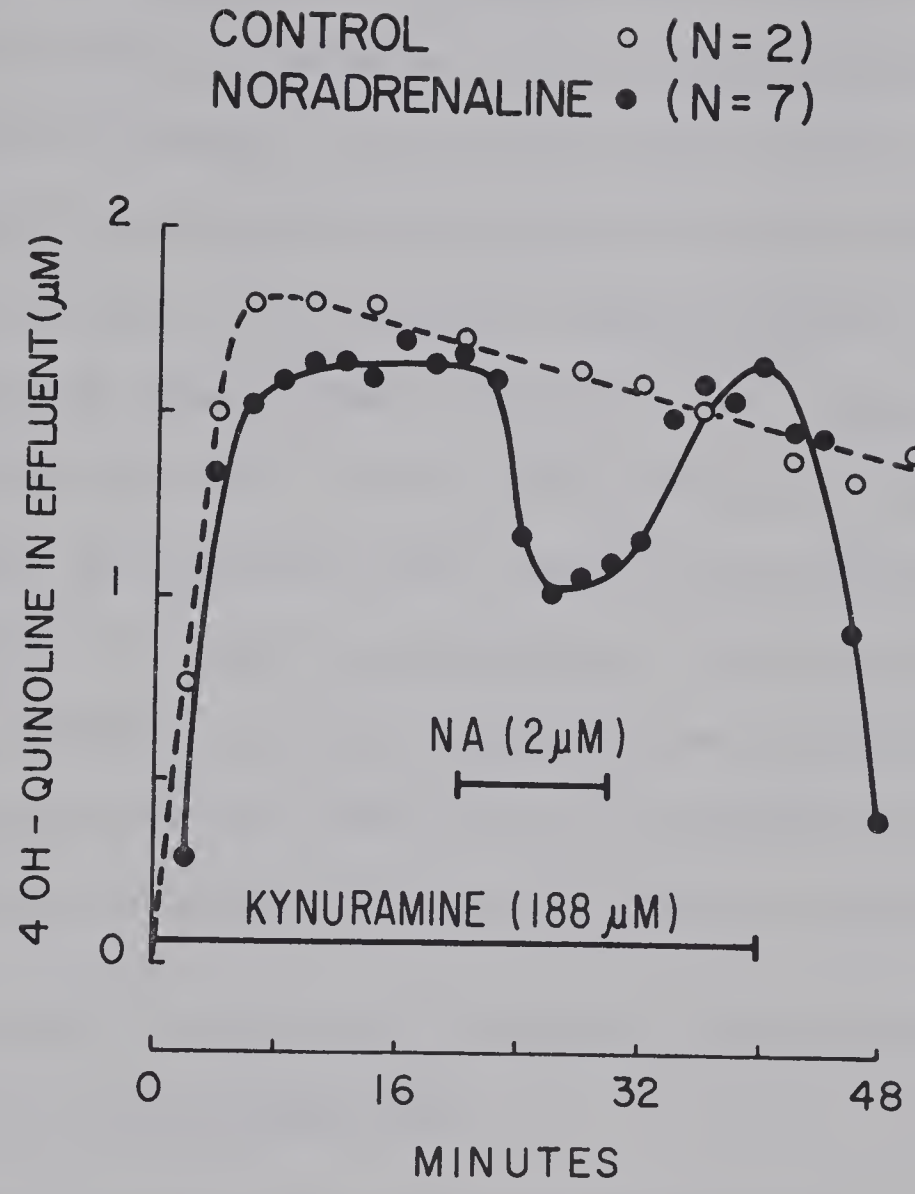


Fig. 5. The influence of noradrenaline on the level of production of 4-OHQ during the perfusion of guinea-pig hearts with kynuramine in a Krebs-bicarbonate medium, compared with a control perfusion of kynuramine over a prolonged time period.

The kynuramine-containing perfusion medium was perfused for 10 minutes before the perfusion medium was changed to one containing DMI (20 μM) as well as kynuramine (188 μM). The simultaneous administration of these two agents was continued for 10 minutes, after which time the noradrenaline infusion was begun as indicated above and maintained for a further 10 minutes. At the end of that period, kynuramine and DMI were infused simultaneously for another 10 minute period. The experiment was concluded with an 8 minute recovery period in which the hearts were perfused with normal Krebs-bicarbonate medium. Figure 6 shows that DMI had no inhibitory effect on the plateau level of 4-OHQ output. The slight increase in the plateau level found in the presence of DMI may be due to blockade of reuptake of spontaneously released noradrenaline resulting in a reduced intracellular level of free noradrenaline. An initial, short duration drop in the 4-OHQ level is seen immediately after commencing the NA infusion, followed by a prompt return to normal levels.

4. The effect of simultaneous infusions of kynuramine and reserpine on the output of 4-hydroxyquinoline.

Figure 7 shows that reserpine (2.17 μM) causes an abrupt fall in the level of 4-OHQ production, which remains at its reduced level until the reserpine infusion is turned off. The 4-OHQ level then returns above the control level.

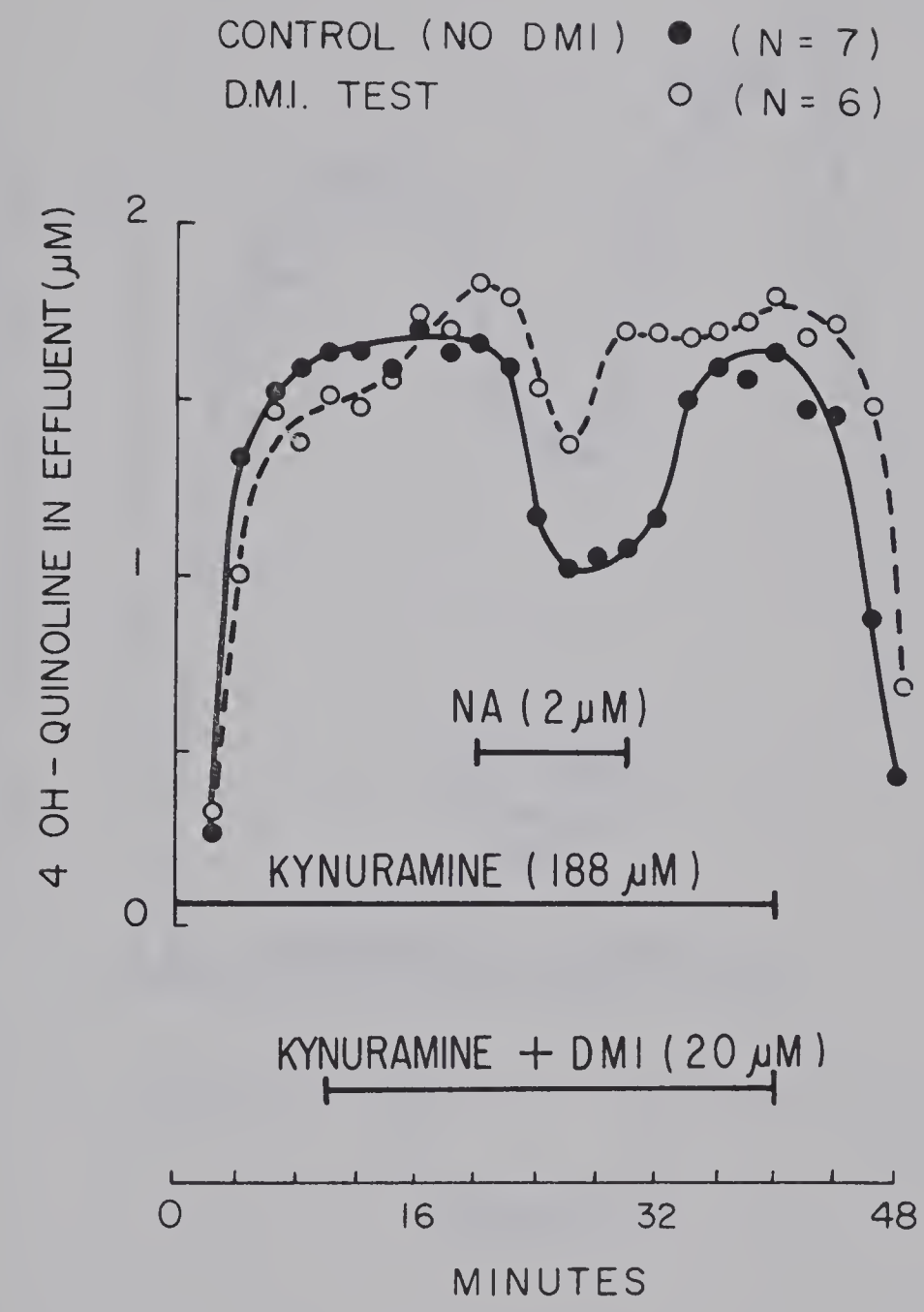


FIG. 6. The influence of desmethylimipramine on the combined infusion of kynuramine and noradrenaline through guinea-pig hearts.

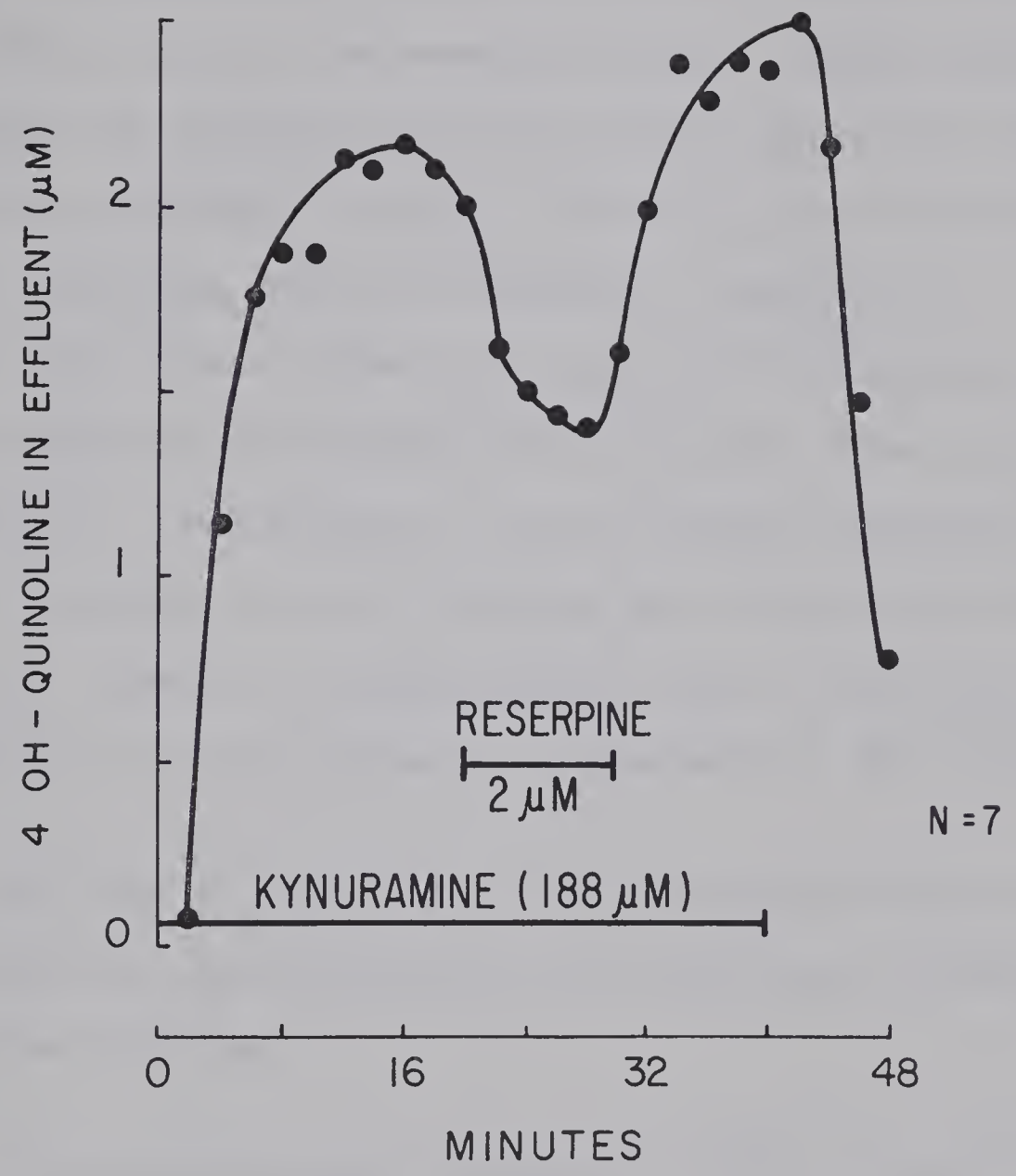


FIG. 7. The influence of reserpine on the level of 4-OHQ production during the perfusion of guinea-pig hearts with kynuramine in a Krebs-bicarbonate medium.

5. The effect of simultaneous infusions of kynuramine and dopamine, tyramine or phenylethylamine on the output of 4-hydroxyquinoline.

Dopamine is a biogenic amine which is taken up into the nerve ending with equal facility to noradrenaline (23, 71) and is deaminated by MAO (43) but has very low vasomotor activity. Figure 8 shows that when dopamine was infused concurrently with kynuramine, the 4-OHQ output falls during the combined infusion. Tyramine is less well taken up into the nerve ending than either noradrenaline or dopamine, but is also deaminated by MAO. Figure 8 shows that tyramine is less effective as a competitor with kynuramine for deamination by MAO than either dopamine or noradrenaline. Phenethylamine is not as readily deaminated by MAO as tyramine or dopamine, nor is it taken up into the nerves as efficiently as they are. However, it is approximately equal in efficiency to tyramine as a competitor for the deamination of kynuramine by MAO (43) (Fig. 8).

6. The effect of pretreatment of guinea-pigs with 6-hydroxydopamine on the output of 4-hydroxyquinoline during simultaneous kynuramine and noradrenaline infusions.

Six guinea-pigs were treated with 6-OHDA as described previously. A similar infusion schedule was performed on the hearts taken from these animals as in other perfusion experiments except that there was a thirty minute period of kynuramine perfusion before the NA was infused. This was done to establish the plateau level of 4-OHQ output for comparison of the 6-OHDA treated animals with the control group.

The results show that the plateau level of 4-OHQ obtained in

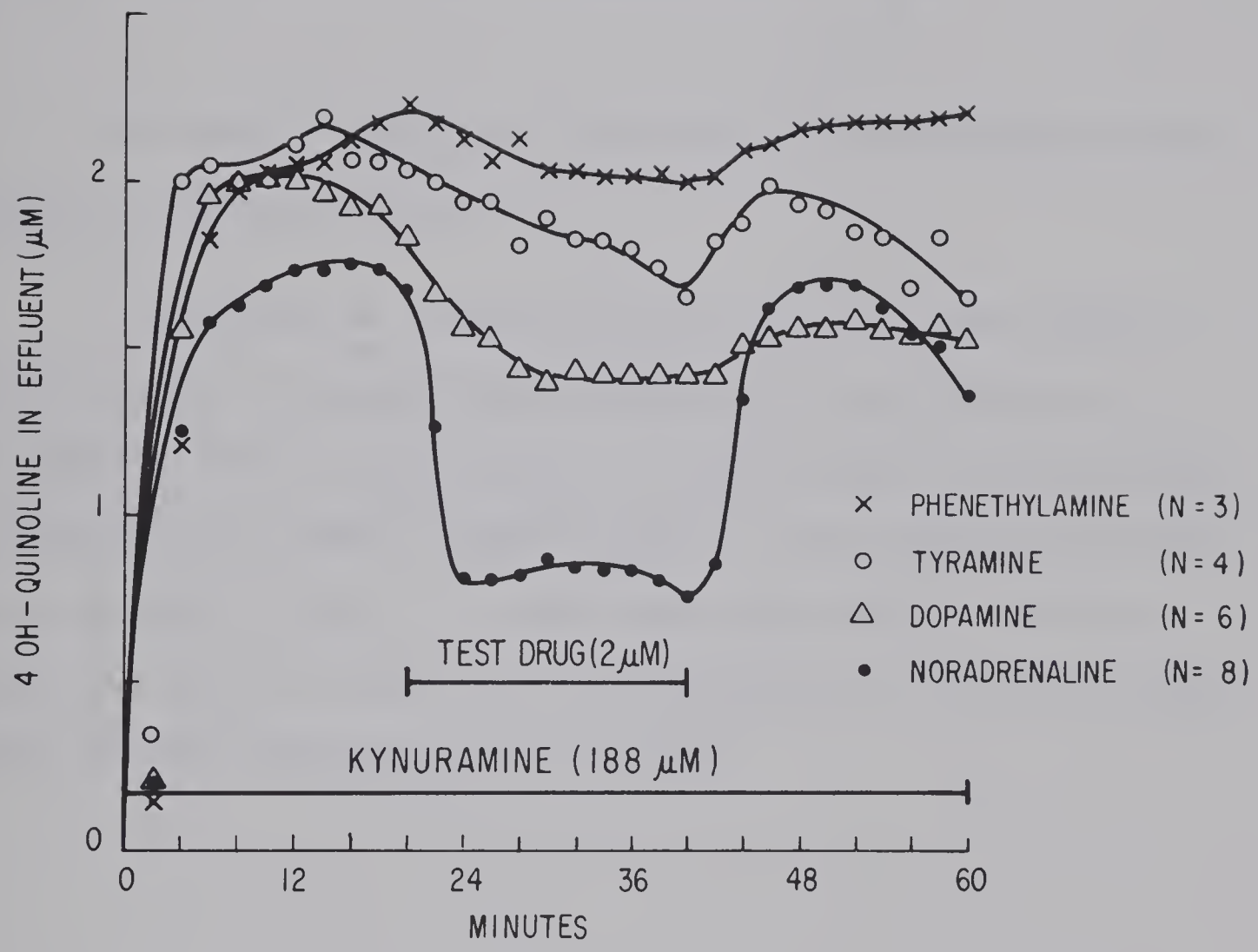


FIG. 8. The influence of phenethylamine, tyramine, dopamine and noradrenaline on the level of 4-OHQ production during the perfusion of guinea-pig hearts with kynuramine in a Krebs-bicarbonate medium.

the control (untreated)animals, is significantly higher ($P < 0.005$) than in the treated animals. However, there is only a small reduction in the percentage fall (49% vs 37%) in the level of 4-OHQ, occurring as a result of NA infusion. These results are presented in Fig. 9.

7. The effect of electrical stimulation on the conversion of kynuramine to 4-hydroxyquinoline.

Stimulation of the heart at the sino-atrial node caused an initial slowing of the heart rate, followed by a rapid increase to a rate greater than the resting level. A typical chart recording during stimulation of the heart is shown in Fig. 10. The results of the MAO assays are shown in Fig. 11. During each three minute stimulation period the level of 4-OHQ output dropped sharply and returned to normal during the rest periods between stimulation.

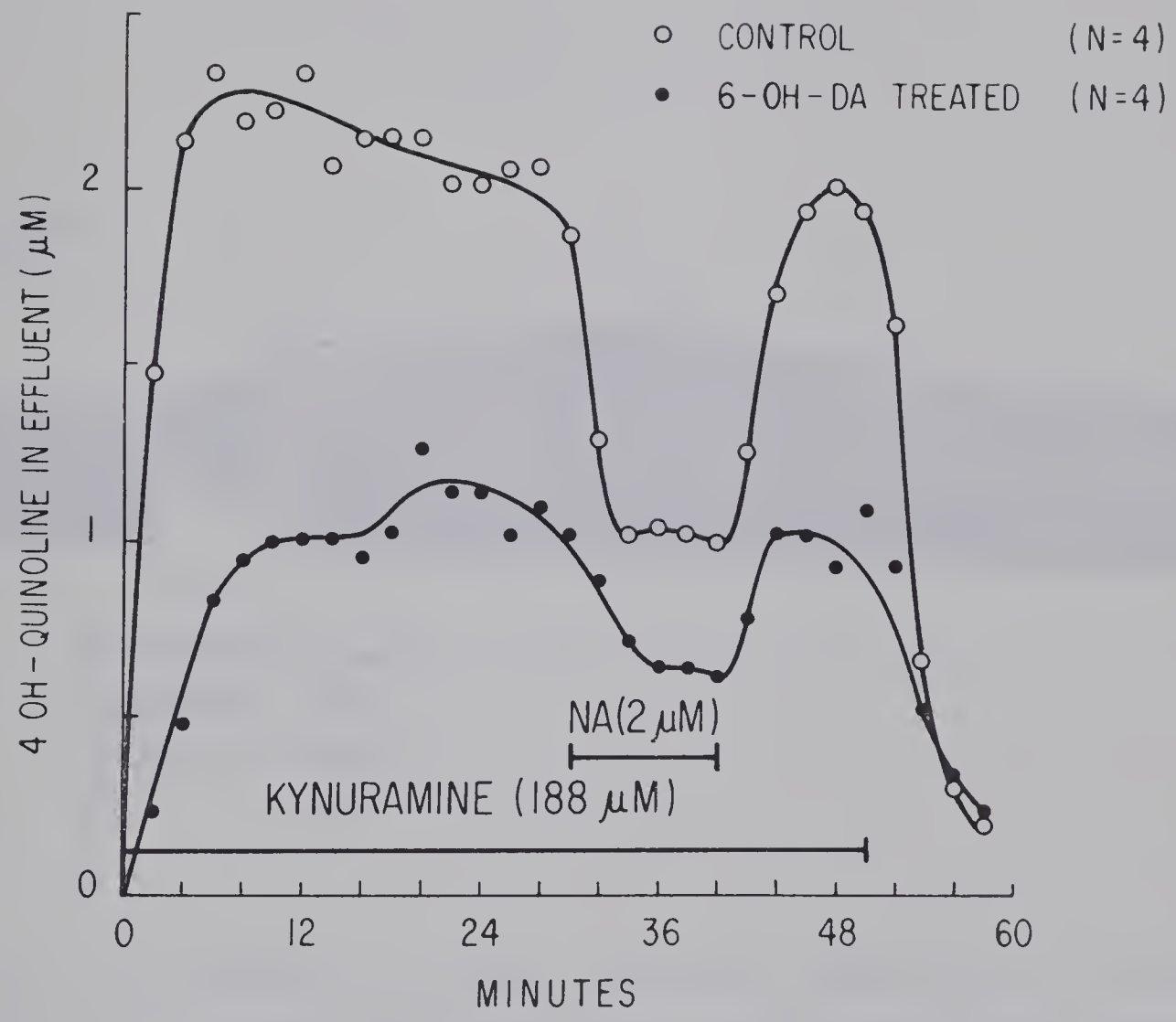


FIG. 9. The effect of pretreatment of guinea-pigs with 6-hydroxydopamine (2 x 100 mg/kg I.P.) on the level of 4-OHQ production during perfusion of the hearts with kynuramine and the influence of noradrenaline infusion on these levels.

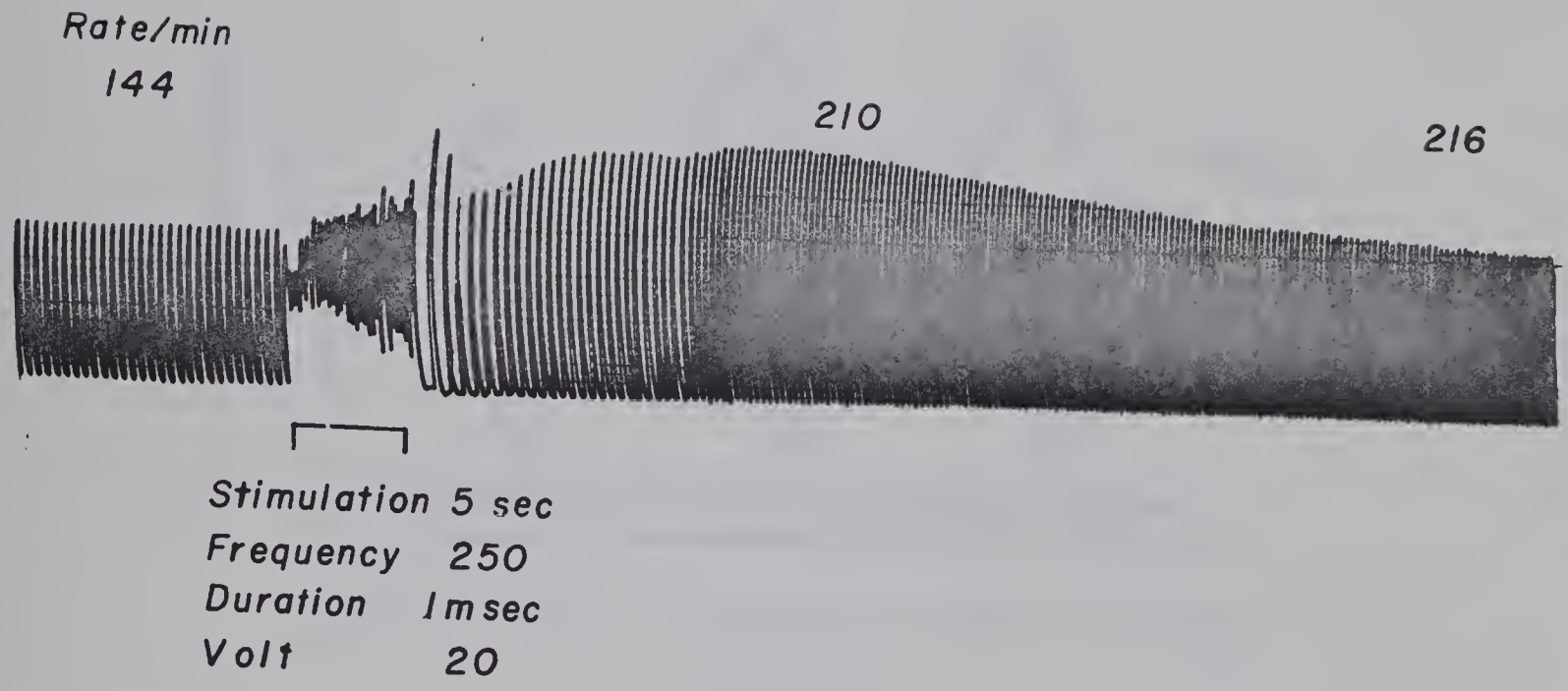


FIG. 10. The effect of direct electrical stimulation of the sino-atrial node of the rabbit heart, on the heart rate, while being perfused by a modified Langendorff method.

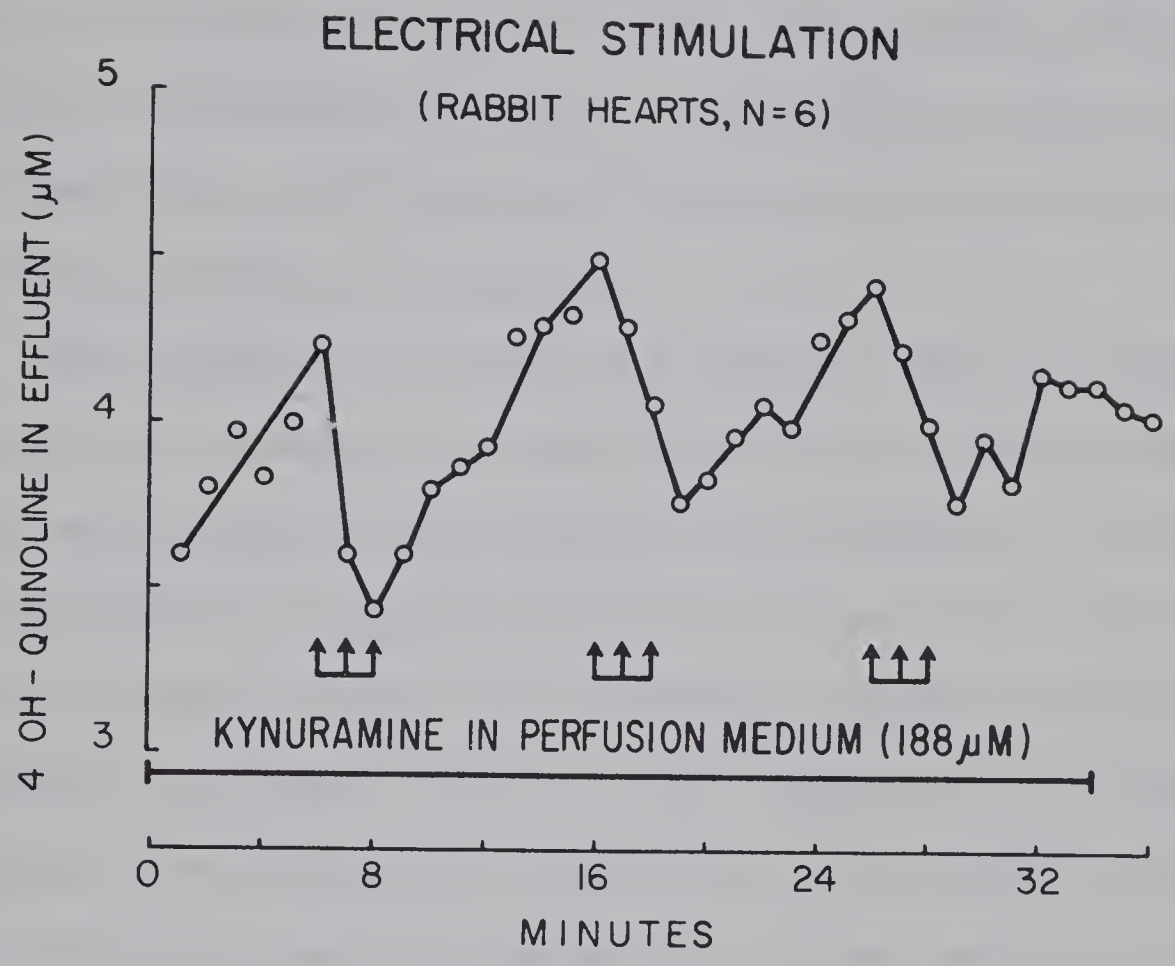


FIG. 11. The effect on 4-OHQ production, of electrical stimulation of the sino-atrial node during perfusion of a rabbit heart with kynuramine. The arrows indicate the electrical stimulation. The characteristics of the stimulation were: strength 20v; frequency 250 per sec; duration 1 msec; the node was stimulated for the first 5 seconds of 3 consecutive minutes.

DISCUSSION

In favour of the intracellular theory of release of noradrenaline from sympathetic nerve endings is the logical assumption that there is an unbound pool of free NA in the nerve endings, through which NA must pass when released from binding by reserpine, sympathomimetic drugs or possibly by nerve stimulation, and through which NA taken up into the nerve ending must pass before binding in the granule.

This assumption is supported by the fact that NA released from perfused hearts by reserpine, is deaminated (58), presumably by MAO located in the mitochondria of the sympathetic nerve endings. In addition, all processes which cause depolarization and release of NA, such as application of cholinergic drugs, excess potassium and nerve stimulation, require calcium for release (8, 36, 37, 56). Hillarp (53, 55) drew an analogy between the stimulation-contraction coupling in muscle cells and a proposed stimulation secretion mechanism in adrenal medulla cells. He suggested that when the chromaffin cells in the adrenal medulla are stimulated by calcium the adenosine triphosphatase (ATPase) associated with the chromaffin granules, perhaps in their membranes, may be activated in some way, enabling it to attack the ATP/4NA storage complex thus freeing the amine, allowing it to diffuse or be transported into the cytoplasm and eventually out of the cell. However, there is still a need for evidence that a free, fluctuating pool of NA exists in nerve endings, before this otherwise attractive hypothesis can be accepted.

The main approach to this problem taken in the present project is based on the MAO assay involving the conversion of the synthetic sub-

strate kynuramine to the fluorescent product, 4-hydroxyquinoline. An in vitro system was used in which the source of MAO was guinea-pig liver tissue, as well as an in vivo guinea-pig heart perfusion system.

Although several distinct MAO enzymes have been isolated from different species and tissues (77), the differences in these enzymes lie mainly in their physical properties. The biochemical activity of the liver and heart enzymes was considered sufficiently similar for comparison of effect in these two systems. MAO is present in much higher quantities in liver tissue than in heart, which made the former tissue a more convenient source of the enzyme for the in vitro studies.

The important concept inherent in the method is that if NA and kynuramine compete for the same position on the enzyme active centre and if for any reason the intracellular concentration of NA rises, then the concentration of 4-OHQ, the product of kynuramine oxidation, will decrease. This hypothesis was validated in the in vitro test in which it was shown that NA, at concentrations of 30μM and greater, inhibits the formation of 4-hydroxyquinoline (Fig. 3).

In the in vivo system it was shown that after the initial perfusion time was completed a slightly declining plateau level of 4-OHQ output could be maintained for a prolonged period in a near steady-state condition (Fig. 5). Simultaneous infusion of sufficient NA to give a final concentration of 2μM in the perfusion fluid caused a sharp fall in the output of 4-OHQ. In vitro, this concentration has no inhibitory effect on 4-OHQ production. The implication from this result is that, since the concentration of NA in the region of the enzyme is probably relatively high, due to its uptake into the nerve, the kynuramine has a greater de-

gree of competition from the NA for active sites on the enzyme. Therefore the amount of 4-OHQ produced is less.

The possibility existed that the infused NA was taken into the nerve cell, as is known to happen physiologically, and that this reuptake mechanism was adding sufficient noradrenaline to the intracellular environment to cause the result seen in Fig. 5. In order to prevent this possibility, desmethylimipramine (DMI) which prevents the reuptake of noradrenaline into the nerve endings, was tested in both in vivo and in vitro test systems. In the in vitro system no inhibitory effect of DMI on the enzymatic production of 4-OHQ was seen (Table I). In vivo, it was found that, at a concentration of 20 μM, DMI did not inhibit the production of 4-OHQ, but may even have potentiated it a little. The potentiation may have been due to a block of the reuptake of NA which is released spontaneously from nerve endings. It may itself be evidence of spontaneous extracellular release. When NA was perfused simultaneously with DMI and kynuramine, there was a short duration drop in 4-OHQ production followed by a prompt rise to normal. This result is in agreement with the findings of Snyder, Fischer and Axelrod, indicating that MAO is found in the nerve endings, since the DMI was shown to prevent the inhibition of the kynuramine deamination by not allowing NA to re-enter the nerve cell, thereby preventing its access to the intracellular MAO.

In order to test for the effect of intracellular release of NA, experiments were carried out using reserpine, an agent which is known to have an effect directly on the storage granules of NA within sympathetic nerve endings (16, 20), and to cause release of the acid metabolites of NA (DOMA) from isolated perfused rat hearts (70). The in vivo experiments

showed rapid fall in the 4-OHQ levels at the onset of the reserpine infusion (2.17 μM), followed immediately by a rise back to normal levels on stopping the reserpine infusion. Since in the in vitro experiments there was no evidence of inhibition of MAO by reserpine itself until the concentration was nearly 50 times that used in the in vivo experiments, these results were taken to indicate that the inhibition seen during infusion of reserpine, was caused indirectly by release of NA from the storage granules into the intracellular environment. The rise in the intracellular level of 'free' NA more readily competes with the kynuramine, thus producing a decrease in level of 4-OHQ produced. The results from the experiments with reserpine are interpreted as evidence that the method gives a valid indication of changes in the intracellular concentration of free NA.

The results of the combined kynuramine and dopamine infusions indicate that the fall in 4-OHQ output which occurs during the simultaneous infusions of kynuramine and noradrenaline, is not due to vasomotor activity. Dopamine has little or no vasomotor activity but is efficiently taken up into the nerve ending and is a good substrate for MAO. The observed fall in 4-OHQ output occurring during the dopamine infusion thus demonstrates an enzymological, rather than a vasomotor effect. Tyramine, which is a good substrate for MAO in vitro, is neither as readily taken up into the nerve endings as noradrenaline or dopamine nor is it as efficient an inhibitor of MAO in the in vivo experiments. This result indicates that the enzyme involved in the system probably has an intraneuronal location.

As the method produced results consistent with the theory for

testing for changes in the intracellular free NA concentration, other procedures which are known to cause the release of noradrenaline were used to further test the system. During perfusion, hearts were stimulated electrically (1), (voltage, 20; frequency, 250/sec; duration, 1 msec; time, 5 sec) in the region of the sino-atrial node. The heart first showed a result of vagal stimulation and then dramatically increased in rate due to the overpowering effect of sympathetic stimulation, with the concurrent release of noradrenaline (Fig. 10). Analysis of the samples collected during the perfusion showed a fall in the 4-OHQ level of the samples taken while the heart was being stimulated (Fig. 11). This data suggests that nerve stimulation releases stored noradrenaline into a free intracellular NA pool, thereby increasing its competitive effect for the MAO and thus reducing the level of 4-OHQ output.

The use of 6-OHDA in the study of mechanisms involving sympathetic nerve endings has been developed during the last few years. Since almost complete chemical sympathectomy can be achieved with this agent, it has been possible to compare the effects of certain procedures in the presence and absence of sympathetic nerves. In order to study the changes in the level of MAO activity after chemical sympathectomy, guinea-pigs and rats were pretreated with 6-OHDA. After 10 days, the hearts, vas deferens and brains were removed from the animals and assayed for both NA content and MAO activity. In both guinea-pigs and rats, reductions of more than 50% in the activity of MAO were measured in the hearts and vas deferens. Between 60 and 80% reductions in NA content or the MAO activity were affected in the brain tissue of the guinea-pigs or rats. This latter result is in agreement with previous discussions (89) in which it was stated that

6-OHDA does not cross the blood-brain barrier. For this reason no fall in the levels of either NA or MAO activity would be expected in the brain tissue since the 6-OHDA was injected intraperitoneally. A decrease in the level of NA in sympathetically innervated tissues after treatment with 6-OHDA, has been taken as a measure of the degeneration of the sympathetic nerves (92). The observation in a drop in MAO activity after 6-OHDA, to an extent of greater than 50%, can be taken as further evidence that a considerable part of the MAO is localized within sympathetic nerves.

In vivo perfusion experiments were performed using hearts from guinea-pigs which had received doses of 6-OHDA. The controls of this series of experiments were those untreated guinea-pigs which were infused with NA, as described in section B, 2 (Results). The plateau level of 4-OHQ output in the treated group was significantly lower than that of the control group ($P < 0.005$). The percentage fall in 4-OHQ level observed during NA infusion is not affected in the treated group as compared to the control group. The lowered plateau level of 4-OHQ in the treated group is consistent with the in vitro observations on the effect of 6-OHDA, (Table II). Sympathectomy causes a degeneration of the sympathetic nerves with a concurrent lowering in the MAO activity available for this oxidative deamination of kynuramine to 4-OHQ, the observed lowering of the 4-OHQ level might be expected. However, since the tissue NA levels are reduced to a similar extent as the MAO activity levels (Table II) after 6-OHDA, and assuming that those nerves which remain after the action of the sympathectomizing agent are able to function normally it is not unlikely that the fall in the level of 4-OHQ is the same in the control and treated animals, when the change is measured in terms

of percentage. In absolute terms the fall in the 6-OHDA treated group was much smaller, being only 41% of that in the control group.

The experiments with 6-OHDA were performed in order to furnish further evidence for the assumption that MAO is confined mainly in the sympathetic nerve endings. Having achieved this aim, the results further substantiate the validity of the method of MAO assay which was used throughout this project. By the use of this assay method considerable evidence has been gathered to show that a free, fluctuating pool of NA exists in sympathetic nerve endings.

SUMMARY AND CONCLUSION

(1) These tests showed that both in vitro and in vivo, noradrenaline can compete with kynuramine for deamination by MAO. This effect can be prevented by DMI in vivo but not in vitro, indicating that in vivo the action is probably dependent upon the uptake and concentration of NA in the sympathetic nerve endings.

(2) Reserpine can cause an inhibition in vivo at a concentration approximately 1/50 of that which is effective in vitro, indicating the action is probably indirect and due to an intraneuronal release of NA.

(3) Dopamine, tyramine and phenethylamine can each cause reductions in the output of 4-OHQ at concentrations far below that at which they have any vasomotor activity, thus reducing the probability that the observed effect is due to vasomotor effect of NA.

The tests also showed that inhibition of the output of 4-OHQ by these agents, was in the order of their ability to release ^{3}HNA from labelled rat hearts, indicating that they probably act at the nerve ending and are dependent upon their uptake into the nerve endings.

(4) Electrical stimulation not only gave evidence of a release of NA from the perfused heart but also a reduction in the output of 4-OHQ indicating the possibility that nerve stimulation may cause an increase in an intraneuronal pool of free NA.

(5) 6-OHDA treatment reduced the content of NA in the heart as well as both the in vitro and in vivo activity of MAO, indicating the MAO is probably associated with the sympathetic nerve ending.

These results indicate that the deamination of kynuramine in the

perfusion fluid was probably due to MAO associated with sympathetic nerve endings and that fluctuations in a pool of NA in the nerve endings can be detected by the use of this experimental procedure.

POSTSCRIPT

The conclusions drawn above indicate that the MAO in heart tissue is largely confined to neuronal tissue and that the method proposed for measuring changes in the pool of free, fluctuating noradrenaline in nerve endings gives reliable results. However, subsequent work in this laboratory has given equivocal results which throw some doubt on the conclusions drawn above. These include the following:

1. Tests for release of NA by depolarization with excess potassium chloride caused a fall in the output of 4-OHQ as expected. However, control tests using equiosmolar quantities of sucrose or sodium chloride as the potassium chloride gave similar, though not as large, falls in the output of 4-OHQ from the kynuramine-infused hearts. Although the reason for this finding has not been determined, it does throw some doubt on the reliability of this test as a measure of fluctuations in free NA, since other tests using hypertonic sucrose and sodium chloride have not caused the release of stored ^{3}HNA.

2. Further tests of the effects of 6-OHDA on the MAO activity and NA content of rat tissues has confirmed the fall in NA observed in the experiments reported here but not that of the MAO activity. This would indicate that further experiments with more samples are necessary before a final conclusion can be drawn regarding the effect of 6-OHDA denervation on MAO activity and location in heart tissue.

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APPENDIX I

PREPARATION OF SOLUTIONS

The perfusion media and all other solutions were prepared with distilled water which had been passed through a deionizer.

(a) Normal Krebs-bicarbonate Solution.

A concentrated salt solution was prepared by dissolving the following mixture of salts in water to produce a total volume of 1 litre:

Sodium Chloride (NaCl)	82.60 grams
Potassium Chloride (KCl)	4.22 grams
Calcium Chloride ($CaCl_2$)	3.36 grams
Potassium Phosphate (KH_2PO_4)	1.94 grams
Magnesium Sulphate ($MgSO_4.7H_2O$)	3.50 grams

Normal Krebs-bicarbonate solution was prepared by mixing 100 mls of the concentrated salt solution, 900 mls of distilled water, 2.15 g dextrose and 192 mls of 1.3 percent solution of sodium bicarbonate. The pH of this solution was 7.6, but was lowered to 7.35 or 7.40 by aeration with a 95% O_2, 5% CO_2 gas mixture.

The millimolar concentrations of the salts in the final perfusion fluid were as follows: NaCl, 118 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM; KH_2PO_4, 1.2 mM; $MgSO_4.7H_2O$, 1.2 mM; $NaHCO_3$, 25 mM; dextrose, 10 mM.

(b) Concentrations of other solutions used in the perfusion experiments.

(i) Kynuramine: 188 µM final concentration in the perfusion fluid.

Stock solution = 10 mg/ml.

Dilute 2.78ml stock solution to 900 mls with Krebs solution.

Perfuse hearts at 6.0 ml/min.

(ii) Noradrenaline: 2 µM final concentration in the perfusion fluid.

Stock solution = 1 mg/ml as base.

Dilute 0.10 mls stock solution to 10 mls with Krebs solution.

Infuse at 0.2 mls/min into the perfusion fluid.

(iii) Reserpine: 2.17 µM final concentration in the perfusion fluid.

Stock solution = 2.5 mg/ml.

Dilute 1 ml stock solution to 6.8 mls.

Infuse at 0.2 mls/min.

(iv) Desmethylimipramine: 20 µM final concentration in the perfusion fluid.

Stock solution = 10 mg/ml.

Dilute 0.27 mls stock solution to 500 mls with Krebs/kynuramine medium.

Perfuse at 6.0 mls/min.

(v) Tyramine: 2 μM final concentration in the perfusion fluid.

Stock solution = 1 mg/ml.

Dilute 1 ml stock solution to 121.5 mls.

Infuse at 0.2 mls/min.

(vi) Dopamine: 2 μM final concentration in the perfusion fluid.

Stock solution = 1 mg/ml.

Dilute 1 ml stock solution to 108.8 mls.

Infuse at 0.2 mls/min.

(vii) Phenylethylamine: 2 μM final concentration in the perfusion fluid.

Stock solution = 1 mg/ml.

Dilute 1 ml stock solution to 137.5 mls.

Infuse at 0.2 mls/min.

(c) Reagents for the in vitro MAO assay.

(i) Trichloroacetic acid: 10% solution in distilled water.

(ii) Tris buffer: 0.5 M at pH 7.4.

Dissolve 15.1 g Tris in 100 mls 1N HCl. Make up to 250 mls with distilled water. Adjust pH if necessary.

(iii) Kynuramine:

Solutions containing: 200 μg/ml

100 μg/ml

50 μg/ml

25 μg/ml

(iv) Noradrenaline:

Solutions containing: 240 µg/ml

120 µg/ml

60 µg/ml

(v) Reserpine:

Solutions containing: 125 µg/ml

62.5 µg/ml

31.25µg/ml

(vi) Desmethylimipramine:

Solutions containing: 600 µg/ml

60 µg/ml

(vii) 4-hydroxyquinoline standards:

10, 20, 30 and 50 µM solutions.

(viii) Enzyme solution prepared as described in Methods.

(d) Procedure for incubations in the in vitro MAO assay system, to test for the inhibitory effect of various agents.

TEST	KYNURAMINE (0.5 mls)	TRIS BUFFER (0.5 mls)	0.01N HCl (1 ml)	TEST DRUG IN 0.01N HCl (1 ml)	ENZYME (1 ml)
Control	50 µg	✓	✓		✓
	25 µg	✓	✓		✓
	12.5 µg	✓	✓		✓
	6.25 µg	✓	✓		✓
Low Conc. Test Drug	50 µg	✓		✓	✓
	25 µg	✓		✓	✓
	12.5 µg	✓		✓	✓
	6.25 µg	✓		✓	✓
2nd Conc. Test Drug	50 µg	✓		✓	✓
	25 µg	✓		✓	✓
	12.5 µg	✓		✓	✓
	6.25 µg	✓		✓	✓
High Conc. Test Drug	50 µg	✓		✓	✓
	25 µg	✓		✓	✓
	12.5 µg	✓		✓	✓
	6.25 µg	✓		✓	✓
Reagent Blank	50 µg	✓	✓		1 ml sucrose
	50 µg	✓	✓		sucrose
Tissue Blank	(0.5 ml) H_2O	✓	✓		✓
	H_2O	✓	✓		✓
Standards 4-OHQ	(0.5 ml) 10 µM	✓	✓		✓
	20 µM	✓	✓		✓
	30 µM	✓	✓		✓
	50 µM	✓	✓		✓

(e) Reagents for catecholamine assay.

(i) Tris Buffer:

0.5 M Tris base adjusted to pH 9 with 2N HCl.

(ii) 0.1 M EDTA:

37.2 g EDTA in 1 M sodium acetate made up to 1 liter in distilled water. Adjust to pH 7 with 5 N NaOH.

(iii) Iodine solution:

1.27 g iodine made up to 100 ml with absolute alcohol.

(iv) Alkaline sulphite:

Prepared fresh daily due to its light sensitivity. One ml of 25% anhydrous sodium sulphite made up to 10 mls with 5 N NaOH.

(v) 5 N Acetic Acid:

143.5 mls glacial acetic acid made up to 500 mls with distilled water.

(vi) 0.05 N $HClO_4$:

8.36 ml made up to 1 litre of distilled water.

(vii) 0.4 N $HClO_4$.

33.5 mls made up to 500 mls of distilled water.

(viii) Noradrenaline stock:

1.22 mg/ml taken as 1 mg/ml free base.

0.0305g made up to 50 mls with 0.05 N $HClO_4$.

Noradrenaline standard:

To make 0.5 µg/ml, dilute 0.1 ml stock to 100 mls with 0.05 N $HClO_4$.

(f) Procedure for alumina activation for noradrenaline assay.

(i) Add 100 g Al_2O_3 to 500 ml 2N HCl in a 1000 ml beaker.

(ii) Cover with a watch glass and heat at 90 - 100°C for 45 min. with continuous and rapid stirring.

(iii) Remove beaker and allow heavier particles to settle for 1½ min.

(iv) Discard distinctly yellow supernatant along with finer particles of Al_2O_3.

(v) Wash precipitate twice with fresh 250 ml portions of 2N HCl at 70°C for 10 min., discarding the supernatant with the finer particles each time.

(vi) Final acid wash: stir Al_2O_3 with 500 ml of 2N HCl at 50°C for 10 min.

(vii) Decant HCl and wash precipitate repeatedly (approx. 20 times) with fresh 200 ml portions of distilled water until a pH of 3.4 is reached, decanting the finer particles each time.

(viii) Finally transfer the $Al_2O_3$to an evaporating dish and heat at 120°C for 1 hour and then at 200°C for 2 hours.

(ix) Store in an incubator at 37°C to keep the powder dry, or in a desiccator under vacuum.

APPENDIX II

TABLE I. The in vitro inhibitory effect of desmethylimipramine on monoamine oxidase activity.

TUBE NO.	KYNURAMINE (µg/ml)	BUFFER	0.01N HCl	DESMETHYLIMIPRAMINE (µg/ml)
1	2.08	✓	✓	-
2	2.08	✓	✓	-
3	2.08	✓		20
4	2.08	✓		20
5	2.08	✓		200
6	2.08	✓		200
7	4.15	✓	✓	-
8	4.15	✓	✓	-
9	4.15	✓		20
10	4.15	✓		20
11	4.15	✓		200
12	4.15	✓		200
13	8.30	✓	✓	-
14	8.30	✓	✓	-
15	8.30	✓		20
16	8.30	✓		20
17	8.30	✓		200
18	8.30	✓		200

Standard tubes were included as indicated in Appendix I. The results from these have been subtracted from the original readings to give the results shown in the table.

ENZYME	4-HYDROXYQUINOLINE (µg/ml)
✓	0.042
✓	
✓	0.042
✓	
✓	0.042
✓	
✓	0.069
✓	
✓	0.078
✓	
✓	0.074
✓	
✓	0.147
✓	
✓	0.147
✓	
✓	0.147
✓	

TABLE II A. The effect of 6-hydroxydopamine pretreatment on the monoamine oxidase activity in rat tissues.

6-OHDA Treatment: 2 doses 100 mg/kg I.P. on day 1 and day 3.
Animals sacrificed on day 10.

Enzyme activity unit (EAU) = fluorescence/g tissue.

RAT TISSUES

TISSUE	TREATMENT	EAU		% REDUCTION WITH TREATMENT
Heart	Control	0.701 0.985 0.765 0.897	0.837	
				60.57%
	6-OHDA	0.406 0.319 0.320 0.278	0.330	
Brain	Control	0.986 1.230 1.497 1.368	1.270	
				8.66%
	6-OHDA	1.276 1.300 0.820 1.240	1.160	
Vas Deferens	Control	0.860 0.941 0.982 0.793	0.890	
				77.0%
	6-OHDA	0.180 0.190 0.290 0.200	0.215	

TABLE II B. The effect of 6-hydroxydopamine pretreatment on the monoamine oxidase activity in guinea-pig tissues.

6-OHDA Treatment: See Table II A.

Enzyme activity unit (EAU) = fluorescence/g tissue.

GUINEA-PIG TISSUES

TISSUE	TREATMENT	EAU		% REDUCTION WITH TREATMENT
Heart	Control	0.79 0.84 1.57 2.20	1.35	
				61.50%
	6-OHDA	0.30 0.40 0.71 0.68	0.52	
Brain	Control	1.68 1.81 2.12 1.88	1.87	
				5.35%
	6-OHDA	1.81 1.91 1.72 1.63	1.77	
Vas Deferens	Control	1.03 1.13 1.02 0.64 1.28	1.02	
				65.69%
	6-OHDA	0.22 0.71 0.27 0.20	0.35	

TABLE III A. The effect of 6-hydroxydopamine pretreatment on the noradrenaline content of rat tissues.

6-OHDA Treatment: See Table II A.

		RAT TISSUE		
TISSUE	TREATMENT	NA (µg/g)		% REDUCTION WITH TREATMENT
Heart	Control	0.430 0.542 o.301 0.631	0.476	
	6-OHDA	0.050 0.142	0.096	79.8%
Brain	Control	0.379 0.378 0.223 0.362	0.336	
	6-OHDA	0.410 0.405	0.407	0.0%
Vas Deferens	Control	2.060 2.120 0.753 0.436	1.340	
	6-OHDA	0.671 0.554	0.610	54.48%

TABLE III B. The effect of 6-hydroxydopamine pretreatment on the noradrenaline content of guinea-pig tissues.

6-OHDA Treatment: See Table II A.

		GUINEA-PIG TISSUE		
TISSUE	TREATMENT	NA(µg/g)		% REDUCTION WITH TREATMENT
Heart	Control	1.170 1.430 0.750 0.680 0.840	0.91	
	6-OHDA	0.580 0.480 0.300 0.270 0.260 0.170 0.270	0.29	68.1%
Brain	Control	0.270 0.387 0.164 0.167 0.158	0.22	
	6-OHDA	0.179 0.270 0.309 0.173 0.180 0.152 0.152	0.21	4.5%
Vas Deferens	Control	1.010 0.525 3.550 2.400 2.960	2.22	
	6-OHDA	2.860 0.078 0.201 0.715 0.640 0.609 0.740	0.50	77.5%

TABLE IV. The in vitro inhibitory effect of noradrenaline on monoamine oxidase activity.

TUBE NO.	KYNURAMINE (µg/ml)	BUFFER	0.01N HCl	NORADRENALINE (µM)
1	1.04	✓	✓	-
2	1.04	✓		30
3	1.04	✓		60
4	1.04	✓		120
5	2.08	✓	✓	-
6	2.08	✓		30
7	2.08	✓		60
8	2.08	✓		120
9	4.15	✓	✓	-
10	4.15	✓		30
11	4.15	✓		60
12	4.15	✓		120
13	8.30	✓	✓	-
14	8.30	✓		30
15	8.30	✓		60
16	8.30	✓		120

Standard tubes were included as indicated in Appendix I. The results from these have been subtracted from the original readings to give the results shown in the table.

Data plotted in Fig. 3.

ENZYME	4-HYDROXYQUINOLINE (1/V x 10^6M)	KYNURAMINE (1/S x 10^4M)
✓	7.75	
✓	9.70	16.0
✓	11.25	
✓	13.60	
✓	4.25	
✓	5.05	8.0
✓	6.05	
✓	7.35	
✓	2.35	
✓	2.75	4.0
✓	3.15	
✓	3.85	
✓	1.40	
✓	1.80	2.0
✓	2.05	
✓	2.20	

TABLE V. The in vitro inhibitory effect of reserpine on monoamine oxidase activity.

TUBE NO.	KYNURAMINE (µg/ml)	BUFFER	0.01N HCl	RESERPINE (µM)
1	1.04	✓	✓	-
2	1.04	✓		50
3	1.04	✓		100
4	1.04	✓		200
5	2.08	✓	✓	-
6	2.08	✓		50
7	2.08	✓		100
8	2.08	✓		200
9	4.15	✓	✓	-
10	4.15	✓		50
11	4.15	✓		100
12	4.15	✓		200
13	8.30	✓	✓	-
14	8.30	✓		50
15	8.30	✓		100
16	8.30	✓		200

Standard tubes were included as indicated in Appendix I. The results from these have been subtracted from the original readings to give the results shown in the table.

Data plotted in Fig. 4.

ENZYME	4-HYDROXYQUINOLINE (1/V x 10^6M)	KYNURAMINE (1/S x 10^4M)
✓	3.60	
✓	3.85	16.0
✓	4.25	
✓	4.65	
✓	2.00	
✓	1.90	8.0
✓	2.15	
✓	2.60	
✓	1.00	
✓	1.10	4.0
✓	1.20	
✓	1.50	
✓	0.45	
✓	0.52	2.0
✓	0.60	
✓	0.70	

TABLE VI. To test the effect of prolonged kynuramine infusion through guinea-pig hearts.

Samples 1-6, taken at 2 minute intervals.

Samples 7-21 taken at 4 minute intervals.

INFUSION	TUBE NO.	HEART 1 RFI	HEART 2 RFI	AVERAGE RFI	4-OHQ (µg/ml)
Kynuramine	1	0.99	0.69	0.84	0.14
	2	1.92	1.80	1.86	0.31
	3	1.74	1.71	1.73	0.29
	4	1.95	1.71	1.83	0.31
	5	1.92	1.65	1.79	0.30
	6	1.98	1.59	1.79	0.30
	7	2.28	1.68	1.98	0.34
	8	2.28	1.56	1.92	0.33
	9	2.16	1.71	1.94	0.33
	10	2.07	1.68	1.88	0.31
	11	1.92	1.62	1.77	0.30
	12	1.89	1.53	1.71	0.29
	13	1.77	1.50	1.64	0.28
	14	1.47	1.26	1.37	0.23
	15	1.53	1.23	1.38	0.23
	16	1.50	0.96	1.23	0.21
	17	1.71	1.23	1.47	0.25
	18	1.59	1.23	1.41	0.24
	19	1.56	1.23	1.40	0.24
	20	1.50	1.26	1.38	0.23
	21	1.47	1.23	1.35	0.23

Data plotted in Fig. 5.

TABLE VII. The influence of noradrenaline infusion (2μM) on the output of 4-hydroxyquinoline during the perfusion of kynuramine through guinea-pig hearts.

INFUSION	TUBE NO.	HEART 1 RFI	HEART 2 RFI	HEART 3 RFI
Kynuramine	1	0.036	0.11	0.14
	2	0.45	0.96	1.53
	3	0.63	1.41	1.82
	4	0.89	1.32	1.55
	5	0.98	1.35	2.20
	6	0.93	1.19	2.40
	7	0.69	1.16	2.60
	8	1.05	1.23	2.90
	9	1.01	1.29	2.40
	10	0.87	1.23	2.70
Kynuramine	11	0.89	0.95	1.76
+	12	0.57	0.93	1.82
Noradrenaline	13	0.54	0.80	1.35
	14	0.57	0.81	1.52
	15	0.62	0.85	1.32
Kynuramine	16	0.68	1.02	1.71
	17	1.01	0.99	2.03
	18	0.86	1.08	1.98
	19	1.07	1.14	2.15
	20	1.20	1.08	2.30
Krebs	21	0.75	1.04	2.10
	22	1.02	1.07	1.83
	23	0.80	0.66	1.04
	24	0.46	0.32	0.49

Data plotted in Fig. 5.

HEART 4 RFI	HEART 5 RFI	HEART 6 RFI	HEART 7 RFI	AVERAGE RFI	4-OHQ (µg/ml)
0.31	0.36	0.28	0.31	0.22	0.04
1.23	1.81	0.99	1.17	1.16	0.20
1.26	1.69	1.11	1.40	1.33	0.23
1.43	1.59	1.32	1.55	1.38	0.24
1.11	1.27	1.43	1.70	1.43	0.24
1.31	1.44	1.10	1.71	1.44	0.24
1.23	1.69	0.90	1.43	1.38	0.24
1.26	1.59	0.84	1.65	1.50	0.25
1.32	1.50	0.87	1.65	1.43	0.24
1.22	1.48	0.84	1.78	1.45	0.24
1.04	1.36	0.84	1.56	1.20	0.20
0.77	1.14	0.59	1.35	1.02	0.17
0.78	1.08	0.59	1.14	0.90	0.15
0.77	1.18	0.65	1.08	0.94	0.16
0.77	1.35	0.74	0.99	0.95	0.16
0.75	1.29	0.75	1.13	1.05	0.17
0.89	1.74	1.05	1.62	1.33	0.23
0.98	1.84	1.14	1.88	1.39	0.24
0.96	1.74	0.78	1.65	1.36	0.23
1.01	1.92	0.95	1.68	1.45	0.24
0.98	1.62	0.90	1.56	1.28	0.22
0.90	1.56	1.05	1.38	1.26	0.21
0.53	0.66	0.78	0.90	0.77	0.13
0.25	0.26	0.42	0.39	1.37	0.06

TABLE VIII. The influence of noradrenaline infusion (2μM) on the output of 4-hydroxyquinoline during the perfusion of kynuramine through guinea-pig hearts. Control data for the desmethylimipramine test.

INFUSION	TUBE NO.	HEART #1 RFI	HEART #2 RFI	HEART #3 RFI
Kynuramine	1	0.017	0.03	0.02
	2	1.13	0.51	0.17
	3	3.15	1.16	1.58
	4	3.25	1.22	1.74
	5	2.80	1.23	1.64
	6	2.55	1.25	1.70
	7	2.75	1.26	1.73
	8	3.00	1.31	1.47
	9	2.35	1.23	1.49
	10	2.50	1.10	1.55
Kynuramine	11	1.80	0.86	1.23
+	12	1.14	0.59	0.86
Noradrenaline	13	1.08	0.63	0.95
	14	1.14	0.62	0.89
	15	1.20	0.66	1.22
Kynuramine	16	1.46	0.78	1.68
	17	4.75	2.27	3.25
	18	2.75	1.52	1.65
	19	2.25	1.61	1.58
	20	2.60	1.53	1.52
Krebs	21	3.00	1.47	1.64
	22	3.05	1.46	0.83
	23	1.50	1.01	0.86
	24	0.45	0.45	0.28

Data plotted in Fig. 6.

HEART #4 RFI	HEART #5 RFI	HEART #6 RFI	HEART #7 RFI	AVERAGE RFI	4-OHQ (µg/ml)
0.02	0.11	0.10	0.07	0.05	0.007
0.73	0.35	0.56	0.39	0.55	0.095
1.77	0.84	1.25	0.99	1.53	0.265
2.66	0.92	1.47	1.32	1.80	0.310
2.25	1.14	1.49	1.05	1.66	0.285
2.35	1.17	1.49	1.17	1.67	0.285
2.35	1.04	1.47	1.32	1.70	0.290
2.35	1.19	1.41	1.43	1.74	0.300
2.25	1.19	1.41	1.46	1.63	0.280
2.15	1.05	1.43	1.47	1.61	0.275
1.56	1.08	1.05	1.43	1.29	0.220
1.08	0.92	0.69	1.14	0.92	0.155
1.10	0.96	0.71	0.69	0.87	0.150
1.16	0.99	0.73	0.68	0.98	0.165
1.16	1.00	0.75	0.69	0.95	0.165
1.35	1.19	0.92	0.77	1.16	0.195
2.27	1.11	1.47	1.28	2.34	0.400
2.70	1.00	1.67	1.44	1.82	0.310
2.60	1.17	1.67	1.47	1.76	0.305
2.40	1.12	1.53	1.44	1.73	0.300
2.30	1.10	1.37	1.41	1.76	0.305
1.95	1.07	1.34	1.35	1.58	0.270
0.92	0.80	1.14	0.90	1.02	0.170
0.47	0.36	0.51	0.42	0.42	0.075

TABLE IX. The influence of desmethylimipramine infusion (20μM) on the combined infusions of noradrenaline (2μM) and kynuramine through guinea-pig hearts.

INFUSION	TUBE NO.	HEART #1 RFI	HEART #2 RFI	HEART #3 RFI
Kynuramine	1	0.22	0.02	0.04
	2	0.79	1.10	0.38
	3	0.93	0.33	0.50
	4	1.00	0.53	0.57
	5	1.10	0.70	0.76
Kynuramine	6	0.98	0.84	0.63
+	7	1.29	0.64	0.48
DMI	8	1.74	0.87	1.08
	9	1.92	0.87	1.20
	10	1.64	0.90	1.38
Kynuramine	11	1.23	0.74	1.28
+	12	1.53	0.69	0.96
DMI	13	1.43	0.62	1.05
+	14	1.38	0.52	1.17
Noradrenaline	15	1.19	0.50	1.11
Kynuramine	16	1.71	0.68	0.79
+	17	1.89	0.95	1.14
DMI	18	1.35	0.65	0.65
	19	1.32	0.74	1.38
	20	1.94	0.86	1.53
Krebs	21	1.38	0.86	1.38
	22	2.26	0.78	1.59
	23	1.62	0.96	1.26
	24	0.74	0.95	0.63

Data plotted in Fig. 6.

HEART #4 RFI	HEART #5 RFI	HEART #6 RFI	AVERAGE RFI	4-OHQ (µg/ml)
0.03	0.02	0.05	0.06	0.007
0.14	0.07	0.09	0.43	0.075
0.28	0.31	0.32	0.45	0.080
0.45	0.41	0.42	0.56	0.095
0.51	0.45	0.47	0.67	0.115
0.47	0.46	0.64	0.67	0.115
0.34	0.55	1.61	0.82	0.140
0.34	0.62	1.34	1.00	0.170
0.52	0.64	1.78	1.16	0.200
0.68	0.58	1.68	1.14	0.195
0.68	0.59	1.65	1.03	0.175
0.49	0.53	1.29	0.92	0.160
0.43	0.74	1.28	0.93	0.160
0.48	0.53	1.35	0.91	0.160
0.46	0.28	1.49	0.84	0.145
0.43	0.39	1.20	0.87	0.150
0.51	0.64	1.53	1.11	0.195
0.61	0.74	1.65	0.94	0.160
0.73	0.73	1.62	1.07	0.185
0.79	0.73	1.67	1.25	0.215
0.75	0.70	1.38	1.08	0.185
0.65	0.64	1.98	1.32	0.225
0.55	0.66	1.91	1.16	0.200
0.39	0.59	1.06	0.73	0.125

TABLE X. The influence of reserpine infusion (2.17μM) on the output of 4-hydroxyquinoline during the perfusion of kynuramine through guinea-pig hearts.

INFUSION	TUBE NO.	HEART #1 RFI	HEART #2 RFI	HEART #3 RFI
Kynuramine	1	0.13	0.058	0.078
	2	0.77	0.88	1.28
	3	0.49	1.43	1.86
	4	0.64	1.41	2.03
	5	0.60	1.62	1.29
	6	0.87	1.61	1.50
	7	1.10	1.86	1.50
	8	1.35	1.98	1.41
	9	1.16	1.56	1.35
	10	0.75	0.99	1.68
Kynuramine	11	0.66	1.56	1.05
+	12	0.72	1.57	1.08
Reserpine	13	0.87	1.24	1.19
	14	0.58	1.04	1.20
	15	0.96	1.05	1.25
Kynuramine	16	1.47	1.83	1.38
	17	1.71	2.20	1.89
	18	1.35	1.77	1.85
	19	1.71	1.89	1.56
	20	1.47	2.06	1.53
Krebs	21	1.83	2.10	2.15
	22	1.77	2.03	1.74
	23	1.05	0.96	1.68
	24	0.47	0.44	0.96

Data plotted in Fig. 7.

HEART #4 RFI	HEART #5 RFI	HEART #6 RFI	HEART #7 RFI	AVERAGE RFI	4-OHQ (µg/ml)
0.06	0.11	0.05	0.09	0.08	0.012
0.78	1.41	0.54	1.22	0.98	0.17
1.32	3.05	0.76	1.89	1.54	0.26
1.43	2.90	0.96	2.06	1.63	0.28
1.50	3.40	1.02	2.01	1.63	0.28
1.55	3.90	1.17	1.98	1.80	0.31
1.49	3.50	1.26	2.20	1.84	0.32
1.61	3.50	1.34	2.05	1.89	0.32
1.70	3.85	1.34	1.95	1.84	0.32
1.61	3.60	1.59	2.09	1.76	0.30
1.19	2.75	0.96	1.71	1.41	0.24
1.10	2.40	0.90	1.38	1.31	0.22
1.10	2.20	0.98	1.32	1.27	0.22
1.08	2.45	0.98	1.41	1.25	0.22
1.16	2.55	1.16	1.53	1.38	0.23
1.14	3.40	1.43	1.67	1.76	0.30
1.50	3.95	1.62	2.01	2.13	0.36
1.50	3.90	1.71	2.15	2.03	0.35
1.62	3.95	1.85	2.40	2.14	0.36
1.50	3.20	1.64	2.25	1.95	0.33
1.35	4.15	1.62	2.35	2.22	0.38
1.92	2.90	1.53	1.52	1.92	0.33
1.20	1.62	0.95	1.59	1.29	0.22
0.53	0.71	0.54	1.10	0.68	0.13

TABLE XI. The influence of tyramine infusion (2μM) on the output of 4-hydroxyquinoline during the perfusion of kynuramine through guinea-pig hearts.

INFUSION	TUBE NO.	HEARTS #1 RFI	#2 RFI	#3 RFI	#4 RFI	AVERAGE RFI	4-OHQ (μg/ml)
Kynuramine	1	3.20	7.3	8.8	8.5	6.95	0.050
	2	18.00	43.0	52.5	46.5	40.00	0.290
	3	24.00	42.0	59.5	42.5	42.00	0.300
	4	26.40	44.5	51.0	42.0	40.98	0.290
	5	25.20	42.5	51.5	44.0	40.80	0.290
	6	25.95	42.5	57.5	45.5	42.86	0.310
	7	24.30	42.5	61.0	46.0	43.45	0.320
	8	25.05	41.5	55.5	45.0	41.76	0.300
	9	25.05	41.5	59.5	45.0	42.76	0.300
	10	24.30	41.5	58.5	44.5	42.20	0.296
Kynuramine	11	22.50	39.5	55.0	44.0	40.25	0.291
+	12	22.80	38.5	53.5	44.0	39.70	0.283
Tyramine	13	23.25	37.5	52.5	55.0	39.56	0.283
	14	20.25	33.5	48.5	44.0	36.56	0.262
	15	21.00	36.5	52.0	43.5	38.25	0.275
	16	19.50	35.5	51.0	42.0	37.00	0.265
	17	19.20	35.0	51.5	41.5	36.80	0.265
	18	19.05	34.5	51.5	41.0	36.51	0.262
	19	18.60	34.0	51.5	36.8	35.23	0.254
	20	19.20	34.0	51.0	32.5	34.18	0.243
Kynuramine	21	18.60	33.5	50.0	45.0	36.77	0.265
	22	19.20	35.0	54.5	48.5	39.30	0.280
	23	19.65	36.5	55.0	50.0	40.29	0.291
	24	19.65	34.5	53.5	50.0	39.41	0.283
	25	18.60	34.5	54.5	49.0	39.15	0.280
	26	18.00	33.0	52.0	49.0	38.00	0.270
	27	18.00	33.0	50.0	48.5	37.38	0.267
	28	18.75	32.5	51.0	43.5	36.44	0.259
	29	18.60	33.0	51.0	47.5	37.53	0.270
	30	19.20	33.0	46.0	43.5	35.43	0.254

Data plotted in Fig. 8.

TABLE XII. The influence of dopamine infusion (2μM) on the output of 4-hydroxyquinoline during the perfusion of kynuramine through guinea-pig hearts.

INFUSION	TUBE NO.	HEART #1 RFI	HEART #2 RFI	HEART #3 RFI
Kynuramine	1	6.2	4.55	4.1
	2	36.0	17.40	18.6
	3	41.5	21.00	21.6
	4	41.5	22.80	21.6
	5	43.0	24.90	21.6
	6	42.5	25.80	22.5
	7	41.0	25.80	21.6
	8	39.0	26.40	21.6
	9	40.0	25.65	21.9
	10	39.0	25.50	21.4
Kynuramine	11	36.0	24.00	19.5
+	12	36.0	25.05	18.0
Dopamine	13	36.0	24.30	15.9
	14	35.5	24.30	12.9
	15	36.0	24.60	13.5
	16	38.5	21.90	14.1
	17	32.5	22.80	15.3
	18	32.5	23.70	15.3
	19	32.0	23.70	15.7
	20	32.0	23.70	15.6
Kynuramine	21	31.5	23.70	15.3
	22	33.0	24.45	15.9
	23	33.5	23.55	15.6
	24	34.0	24.60	15.9
	25	34.0	23.55	16.2
	26	34.5	24.30	16.3
	27	33.0	22.80	16.2
	28	33.0	23.00	16.2
	29	34.5	23.10	16.8
	30	33.0	23.25	16.5

Data plotted in Fig. 8.

HEART #4 RFI	HEART #5 RFI	HEART #6 RFI	AVERAGE RFI	4-OHQ (µg/ml)
3.0	5.3	6.5	4.97	0.030
26.4	44.0	49.0	31.90	0.228
37.5	57.5	59.5	39.77	0.286
45.0	61.5	50.5	40.48	0.291
47.5	62.5	46.0	40.90	0.294
50.5	59.0	40.0	40.55	0.291
51.0	58.0	40.0	59.70	0.286
49.5	57.5	37.5	38.60	0.278
49.5	55.5	36.5	38.60	0.278
49.0	51.0	32.5	37.15	0.265
45.5	44.0	26.0	33.66	0.241
43.0	43.5	25.0	31.84	0.228
41.5	41.5	25.0	31.00	0.222
41.0	42.5	24.5	29.95	0.214
40.5	42.5	24.5	29.30	0.206
40.5	40.0	24.5	30.33	0.214
39.0	40.0	24.5	29.02	0.209
36.5	41.0	25.5	28.92	0.206
37.0	40.5	26.5	29.35	0.209
37.5	40.5	26.5	29.30	0.209
38.5	40.5	27.0	29.41	0.209
42.5	42.0	27.5	31.06	0.222
44.0	44.0	27.5	31.36	0.225
45.0	44.5	29.5	32.25	0.230
45.0	45.0	29.5	32.02	0.228
45.0	44.0	30.0	32.36	0.230
43.5	43.0	30.0	31.41	0.225
43.5	42.5	29.5	31.28	0.222
43.5	44.5	29.5	31.93	0.228
41.0	43.5	29.5	31.13	0.222

TABLE XIII. The influence of phenethylamine infusion (2μM) on the output of 4-hydroxyquinoline during the perfusion of kynuramine through guinea-pig hearts.

INFUSION	TUBE NO.	HEARTS #1 RFI	#2 RFI	#3 RFI	AVERAGE RFI	4-OHQ (μg/ml)
Kynuramine	1	6.6	0.94	5.0	4.2	0.026
	2	50.0	6.4	37.5	31.3	0.175
	3	60.5	34.5	47.5	47.5	0.267
	4	61.0	40.5	50.0	50.5	0.286
	5	57.0	47.5	51.0	51.8	0.294
	6	57.5	49.5	52.0	53.0	0.299
	7	56.0	52.5	52.0	53.5	0.300
	8	56.5	53.0	54.0	54.5	0.310
	9	56.5	54.0	55.5	55.3	0.315
	10	55.0	57.5	60.0	57.5	0.325
Kynuramine + PEA	11	52.5	57.0	56.0	55.2	0.315
	12	48.5	57.5	56.0	54.0	0.307
	13	49.0	55.0	55.5	53.2	0.299
	14	48.5	57.0	57.0	54.2	0.310
	15	47.5	56.0	55.0	52.8	0.296
	16	47.0	56.5	54.0	52.5	0.296
	17	46.0	54.5	55.0	51.8	0.294
	18	46.0	55.5	53.5	51.6	0.294
	19	46.0	57.0	55.0	52.6	0.296
	20	44.5	56.5	52.5	51.2	0.291
Kynuramine	21	46.0	57.0	52.5	51.8	0.294
	22	48.0	59.5	54.5	54.0	0.307
	23	48.0	59.5	55.0	54.2	0.310
	24	48.5	61.5	57.0	55.5	0.315
	25	48.0	61.5	55.0	54.8	0.312
	26	48.0	62.5	56.5	55.7	0.315
	27	49.0	64.0	57.0	56.7	0.318
	28	50.0	65.0	55.0	56.7	0.318
	29	49.0	66.0	54.0	56.3	0.320
	30	48.5	66.5	55.0	56.7	0.323

Data plotted in Fig. 8.

TABLE XIV. The influence of noradrenaline infusion (2μM) on the output of 4-hydroxyquinoline during the perfusion of kynuramine through guinea-pig hearts.

INFUSION	TUBE NO.	HEART 1 RFI	HEART 2 RFI	HEART 3 RFI	HEART 4 RFI
Kynuramine	1	4.8	3.1	8.7	6.4
	2	27.0	21.6	40.5	37.5
	3	31.5	32.0	47.0	36.5
	4	32.0	36.0	52.0	35.5
	5	31.5	41.0	54.5	37.0
	6	30.5	42.5	57.5	38.0
	7	29.5	43.5	57.0	38.5
	8	29.5	42.5	56.0	39.0
	9	28.0	41.0	54.0	39.5
	10	27.0	40.5	53.0	38.0
Kynuramine	11	17.8	36.0	36.5	29.0
+	12	11.8	25.5	25.0	21.5
Noradrenaline	13	12.5	26.0	24.5	20.5
	14	14.1	26.0	25.5	21.0
	15	16.0	25.0	27.0	21.5
	16	17.1	26.0	27.5	22.0
	17	15.6	24.5	25.0	20.0
	18	15.7	23.5	25.0	20.0
	19	16.0	20.0	20.5	21.0
	20	15.3	18.1	21.0	22.5
Kynuramine	21	17.4	20.7	28.5	23.0
	22	23.8	23.2	39.5	32.0
	23	27.1	26.7	46.0	39.0
	24	28.5	29.0	48.0	37.0
	25	29.5	29.0	48.0	35.5
	26	28.5	28.5	48.0	34.5
	27	27.5	28.5	47.5	32.5
	28	27.0	28.0	46.0	29.5
	29	27.0	27.5	45.0	30.0
	30	27.5	26.5	45.0	28.5

Data plotted in Fig. 8.

HEART 5 RFI	HEART 6 RFI	HEART 7 RFI	HEART 8 RFI	AVERAGE RFI	4-OHQ (µg/ml)
6.0	4.1	6.1	5.0	5.5	0.029
46.0	25.5	42.0	27.0	33.4	0.185
57.5	32.0	50.5	38.5	40.7	0.230
46.5	32.5	52.0	42.0	41.1	0.233
58.5	33.0	50.0	45.0	43.8	0.246
59.0	32.5	50.0	44.0	44.3	0.251
58.5	31.5	47.5	47.0	44.1	0.249
58.5	30.5	48.0	51.0	45.1	0.254
58.0	31.5	47.5	56.0	44.4	0.251
57.5	30.5	44.5	54.0	43.1	0.244
46.0	23.5	31.5	45.5	33.2	0.185
27.0	15.9	19.8	26.5	21.6	0.119
23.5	13.6	19.5	23.5	20.5	0.116
25.5	12.6	20.1	25.0	21.2	0.119
27.0	12.4	24.5	25.5	22.4	0.127
27.0	12.6	21.7	23.5	22.2	0.124
26.5	12.6	20.1	26.0	21.3	0.122
29.5	14.7	20.4	24.5	21.7	0.122
27.5	12.9	21.6	25.5	20.6	0.116
27.5	12.1	19.9	23.0	19.9	0.111
29.0	11.4	22.0	26.5	22.3	0.124
51.0	15.1	40.5	47.5	34.1	0.193
66.5	18.9	47.0	57.5	41.1	0.232
70.0	20.4	50.5	56.5	42.5	0.241
70.5	21.0	50.0	55.5	42.4	0.241
70.0	22.2	50.0	55.5	42.2	0.241
70.5	22.6	49.0	54.5	41.6	0.235
69.5	22.0	44.5	52.0	39.8	0.225
68.5	21.4	44.0	50.5	39.2	0.222
64.5	21.0	44.0	44.5	37.7	0.201

TABLE XV. The influence of noradrenaline infusion (2 μM) on the output of 4-hydroxyquinolien during the perfusion of kynuramine through guinea-pig hearts. Control data for the 6-hydroxydopamine pretreatment test.

INFUSION	TUBE NO.	HEART #1 RFI	HEART #2 RFI	HEART #3 RFI	HEART #4 RFI
Kynuramine	1	2.01	1.59	1.32	3.60
	2	3.40	2.75	1.83	5.00
	3	3.75	2.90	1.88	5.30
	4	3.40	2.95	1.92	4.90
	5	3.40	3.00	2.04	4.95
	6	3.35	3.00	2.15	5.40
	7	3.35	2.95	1.77	4.40
	8	3.25	2.95	1.98	4.80
	9	3.25	2.85	1.95	4.95
	10	3.25	2.80	2.03	4.85
	11	3.20	2.80	1.77	4.25
	12	3.30	2.85	1.92	4.20
	13	3.20	2.90	1.95	4.40
	14	3.15	2.90	2.06	4.40
	15	2.70	2.60	1.98	3.95
Kynuramine + Noradrenaline	16	2.04	1.95	1.20	2.55
	17	1.64	1.68	0.99	1.82
	18	1.68	1.65	1.04	1.97
	19	1.53	1.53	1.16	1.95
	20	1.62	1.59	1.04	1.80
Kynuramine	21	1.92	1.91	1.37	2.23
	22	2.50	2.50	1.73	3.80
	23	2.70	2.60	1.91	4.55
	24	2.50	2.50	2.01	5.10
	25	2.50	2.50	1.92	4.70
Krebs	26	2.07	2.01	1.65	3.95
	27	0.95	0.93	0.79	1.22
	28	0.42	0.40	0.45	0.47
	29	0.23	0.23	0.22	0.25

Data plotted in Fig. 9.

AVERAGE RFI	4-OHQ µg/ml
2.13	0.215
3.25	0.315
3.46	0.340
3.29	0.320
3.35	0.325
3.47	0.340
3.12	0.300
3.25	0.315
3.25	0.315
3.23	0.315
3.01	0.295
3.04	0.295
3.14	0.300
3.13	0.300
2.81	0.275
1.94	0.190
1.53	0.150
1.58	0.155
1.54	0.150
1.51	0.145
1.85	0.185
2.63	0.250
2.94	0.285
3.04	0.295
2.91	0.295
2.42	0.235
0.97	0.095
0.44	0.045
0.23	0.030

TABLE XVI. The influence of noradrenaline infusion (2 μM) on the output of 4-hydroxyquinoline during the perfusion of kynuramine through hearts taken from guinea-pigs which had been treated with 6-hydroxydopamine (2 x 100 mg/kg) and sacrificed 10 days after treatment.

INFUSION	TUBE NO.	HEART #1 RFI	HEART #2 RFI	HEART #3 RFI	HEART #4 RFI
Kynuramine	1	0.250	0.42	0.410	0.222
	2	0.690	0.68	0.975	0.485
	3	0.825	2.40	1.200	0.425
	4	0.975	2.90	1.230	0.520
	5	0.975	3.20	1.245	0.495
	6	1.095	3.20	1.230	0.455
	7	1.050	3.35	1.170	0.400
	8	1.080	3.20	1.115	0.370
	9	1.080	3.10	1.125	0.840
	10	1.095	3.20	1.125	2.100
	11	1.095	3.00	1.065	1.965
	12	1.110	3.00	1.005	1.860
	13	1.080	2.85	0.990	1.245
	14	1.050	2.80	0.960	1.620
	15	1.080	2.35	0.960	1.800
Kynuramine + Noradrenaline	16	0.810	1.86	0.885	1.680
	17	0.555	1.48	0.765	1.455
	18	0.600	1.47	0.720	1.260
	19	0.590	1.47	0.720	1.245
	20	0.590	1.29	0.705	1.290
Kynuramine	21	0.750	1.65	0.735	1.590
	22	1.010	3.05	0.720	1.320
	23	1.260	2.70	0.780	1.335
	24	1.290	2.11	0.750	1.365
	25	0.990	2.79	0.750	2.130
Krebs	26	1.010	1.89	0.840	1.680
	27	0.720	0.70	0.645	1.050
	28	0.375	0.41	0.540	0.640
	29	0.245	0.24	0.385	0.420

Data plotted in Fig. 9.

AVERAGE RFI	4-OHQ (µg/ml)
0.33	0.035
0.71	0.070
1.21	0.120
1.41	0.135
1.48	0.145
1.49	0.145
1.49	0.145
1.44	0.140
1.54	0.150
1.88	0.185
1.78	0.170
1.74	0.170
1.54	0.150
1.61	0.160
1.55	0.150
1.31	0.130
1.07	0.105
1.01	0.100
1.01	0.100
0.97	0.095
1.18	0.115
1.52	0.150
1.52	0.150
1.38	0.135
1.66	0.160
1.35	0.135
0.78	0.075
0.49	0.050
0.32	0.035

TABLE XVII. The influence of direct electrical stimulation of the sino-atrial node of rabbit hearts, on the output of 4-hydroxyquinoline during perfusion with kynuramine.

INFUSION	TUBE NO.	HEART #1 RFI	HEART #2 RFI	HEART #3 RFI
Kynuramine	1	0.01	0.01	0.02
	2	0.02	0.01	0.03
	3	0.20	0.13	0.19
	4	0.41	0.41	0.36
	5	0.57	0.59	0.58
	6	0.60	0.76	0.62
	7	0.65	0.86	0.65
	8	0.67	0.75	0.65
	9	0.71	0.84	0.72
	10	0.76	1.08	0.73
Stimulation	11	0.61	0.83	0.68
	12	0.57	0.59	0.66
	13	0.69	0.66	0.67
Recovery	14	0.69	0.65	0.75
	15	0.69	0.77	0.74
	16	0.69	0.69	0.79
	17	0.75	0.93	0.88
	18	0.72	0.99	0.84
	19	0.69	1.02	0.94
	20	0.69	1.10	1.07
Stimulation	21	0.70	0.96	1.02
	22	0.65	0.81	0.78
	23	0.61	0.74	0.71
Recovery	24	0.65	0.77	0.71
	25	0.67	0.77	0.72
	26	0.66	0.93	0.71
	27	0.66	0.78	0.71
	28	0.69	1.02	0.72
	29	0.67	0.99	0.78
	30	0.69	1.08	0.81
Stimulation	31	0.62	0.96	0.83
	32	0.65	0.87	0.77
	33	0.66	0.72	0.74
Recovery	34	0.66	0.74	0.69
	35	0.67	0.78	0.62
	36	0.66	1.02	0.69
	37	0.65	0.99	0.74
	38	0.63	0.90	0.74
	39	0.60	0.93	0.65
	40	0.60	0.96	0.60
Krebs	41	0.54	0.96	0.60
	42	0.40	0.61	—
	43	0.31	0.60	0.55
	44	0.29	0.54	0.42
	45	0.20	0.44	0.34

Samples taken at 1 minute intervals.
Data plotted in Fig. 11.

HEART #4 RFI	HEART #5 RFI	HEART #6 RFI	AVERAGE RFI	4-OHQ (µg/ml)
0.02	0.01	0.03	0.02	0.001
0.02	0.17	0.05	0.05	0.007
0.27	0.35	0.24	0.23	0.037
0.68	0.43	0.38	0.45	0.080
0.96	0.44	0.45	0.59	0.100
1.07	0.46	0.45	0.66	0.115
1.13	0.46	0.44	0.69	0.120
0.99	0.46	0.50	0.67	0.115
1.07	0.44	0.47	0.71	0.120
1.14	0.42	0.46	0.77	0.130
0.87	0.39	0.48	0.64	0.110
0.71	0.37	0.44	0.56	0.095
0.72	0.42	0.31	0.59	0.100
0.93	0.43	0.42	0.65	0.115
0.99	0.39	0.43	0.69	0.120
1.13	0.38	0.51	0.70	0.120
1.17	0.44	0.49	0.78	0.135
1.20	0.45	0.48	0.78	0.135
1.17	0.46	0.44	0.79	0.135
1.22	0.48	0.47	0.84	0.145
1.01	0.48	0.45	0.77	0.135
1.17	0.45	0.44	0.72	0.120
0.95	0.42	0.42	0.64	0.110
0.96	0.46	0.48	0.67	0.115
1.08	0.46	0.49	0.70	0.120
1.13	0.47	0.54	0.74	0.125
1.14	0.47	0.48	0.71	0.120
1.26	0.49	0.50	0.78	0.135
1.40	0.48	0.51	0.81	0.140
1.35	0.48	0.52	0.82	0.140
1.23	0.48	0.54	0.78	0.135
1.07	0.44	0.40	0.70	0.120
0.96	0.36	0.38	0.64	0.110
1.29	0.35	0.42	0.69	0.120
1.10	0.40	0.44	0.68	0.120
1.17	0.45	0.46	0.76	0.135
1.14	0.44	0.40	0.73	0.125
1.23	0.47	0.35	0.72	0.125
1.23	0.47	0.37	0.71	0.120
1.26	0.40	0.36	0.70	0.120
1.04	0.36	0.08	0.60	0.105
0.75	0.32	0.08	0.43	0.075
0.56	0.27	0.06	0.39	0.070
0.32	0.29	0.06	0.32	0.055
0.24	0.21	0.05	0.25	0.045